

INVESTIGATION OF THE EFFECTS OF LONG NON-CODING RNAs UNDER
DROUGHT STRESS ON TOMATO (*Solanum lycopersicum*)



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INVESTIGATION OF THE EFFECTS OF LONG NON-CODING RNAs UNDER
DROUGHT STRESS ON TOMATO (*Solanum lycopersicum*)

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ABSTRACT

INVESTIGATION OF THE EFFECTS OF LONG NON-CODING RNAs UNDER DROUGHT STRESS ON TOMATO (*Solanum lycopersicum*)

Long non-coding RNAs (lncRNAs) are essential in realizing biological functions like responding to environmental stresses; these functions include plant development, growth, and drought. Tomatoes are economically significant and are rich in vitamins A and C. The tomato (*Solanum Lycopersicum*), widely consumed globally, is a suitable organism for understanding stress mechanisms. Its genome has been fully sequenced, and it is a physiologically well-characterized plant, both from growth and fruit production aspects. However, although studies have been performed regarding lncRNAs during ripening, little is known about non-protein-coding RNAs during growth and stress conditions. This research aims to determine the long non-coding RNAs by sequencing the whole transcriptome for analysis with the NGS method at the conclusion of the drought stress application to the tomato plant. In this research, Falcon and SC2121 tomato cultivars were selected.

The putative roles of the lncRNAs under drought stress were evaluated in this study by applying molecular and physiological experiments. Shoot, root lengths, water content, soluble protein content, and malondialdehyde analyses were measured to determine the impacts of drought stress on the two different tomato varieties. The function of drought-induced lncRNAs were identified by comparative transcriptomic analyses, and then roles of putative drought stress-induced lncRNAs functions were analysed at the genome-wide levels. By high-throughput NGS sequencing, 269 putative lncRNAs were identified here. 124 up- and 145 down-regulated lncRNAs were differentially expressed in Falcon and SC2121. As a result of the data obtained, lncRNAs affecting the response mechanism to drought stress was determined, and new methods will be established to increase product yield. The information obtained from this study will help us comprehend the roles of lncRNAs in drought stress and may be helpful in the process of growing tolerant vegetables in the future.

ÖZET

KURAKLIK STRESİ ALTINDAKİ DOMATESLERDE (*Solanum lycopersicum*) UZUN KODLAMA YAPMAYAN RNA'LARIN ETKİLERİNİN ARAŞTIRILMASI

Uzun kodlamayan RNA'lar (lncRNA'lar), bitki gelişimi, büyüme ve kuraklık gibi çevresel streslere yanıt verme gibi biyolojik işlevlerin gerçekleştirilmesinde önemli bir rol oynar. Domates bitkisi ekonomik olarak önemlidir ve A ve C vitaminleri bakımından zengindir. Dünyada yaygın olarak tüketilen domates (*Solanum Lycopersicum*), stres mekanizmalarını anlamak için iyi bir organizmadır. Genomu tamamen dizilenmiştir ve hem büyüme hem de meyve üretimi açısından fizyolojik olarak iyi karakterize edilmiş bir bitkidir. Bununla birlikte, meyve olgunlaşma sırasında lncRNA'larla ilgili çalışmalar yapılmış olmasına rağmen, büyüme ve stres koşulları sırasında protein kodlamayan RNA'lar hakkında çok az şey bilinmektedir. Bu araştırma, domates bitkisine kuraklık stresi uygulamasından sonra NGS yöntemiyle analiz için tüm transkriptomu dizileyerek uzun kodlamayan RNA'ları belirlemeyi amaçlamaktadır. Bu çalışmada Falcon ve SC2121 domates türleri seçilmiştir.. Bu çalışmada lncRNA'ların kuraklık stresi altındaki etkileri moleküler ve fizyolojik deneyler uygulanarak değerlendirilmiştir. Kuraklık stresinin iki farklı domates çeşidi üzerindeki etkilerini belirlemek için sürgün, kök uzunlukları, su içeriği, çözünür protein içeriği ve malondialdehit analizleri ölçülmüştür. Kuraklığın neden olduğu lncRNA'ların işlevi, karşılaştırmalı transkriptomik analizlerle tanımlandı ve ardından varsayılan kuraklık stresinin neden olduğu lncRNA'ların işlevlerinin rolleri, genom çapında seviyelerde analiz edildi. Yüksek verimli NG dizilemesi ile Falcon ve SC2121 türlerinde, 269 varsayılan lncRNA tanımlandı. Bunlardan, 124 yukarı ve 145 aşağı regüle lncRNA tespit edildi. Elde edilen veriler sonucunda kuraklık stresine tepki mekanizmasını etkileyen lncRNA'lar belirlenmiş olup, ürün verimini arttırmak için yeni yöntemler oluşturulacaktır. Bu çalışmadan elde edilen bilgiler, lncRNA'ların kuraklık stresindeki rollerini anlamamıza ve gelecekte toleranslı sebzelerin yetiştirilmesinde yardımcı olabilir.

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LIST OF SYMBOLS/ABBREVIATIONS

AAC1	Acetyl-CoA carboxylase
ABA	Abcistic acid
APX	Ascorbate peroxidase
AlCl ₃	Aluminum chloride
asRNAs	Antisense ribonucleic acid
ATP	Adenosine tri-phosphate
BSA	Bovine serum albumin
Ca	Calcium
CAT	Catalase
CDK	Encocycle-linked kinases
cDNA	Complementary DNA
Cl	Chlorine
CO ₂	Carbondioxide
CTP	Cytidine triphosphate
CYC	Endocycle-linked cyclins
DGDG	Digalactosyldiacylglycerol
DH ₂ O	Distilled water
DNA	Deoxyribo nucleic acid
DNase	Deoxyribo nuclease
eRNAs	Enhancer ribonucleic acid
H ₂ O ₂	Hydrogen peroxide
hc-siRNAs	Heterochromatic siRNAs
K	Potassium
LncRNA	Long non-coding ribonucleic acid
Na	Sodium
MDA	Malondialdehyde
MgCl ₂	Magnesium chloride
MGDG	Monogalactosyldiacylglycerol
miRNA	Micro ribonucleic acid
mM	Millimolar
NAD	Nicotinamide adenine dinucleotide

NADP	Nicotinamide adenine dinucleotide phosphate
NGS	Next generation sequencing
NaCl	Sodium chloride
ncRNA _s	Non-coding RNAs
PCR	Polymerase chain reaction
piRNAs	Piwi-associated RNAs
qPCR	Quantitative polymerase chain reaction
RBP _s	Ribonucleic acid binding protein
RIN	RNA integrity number
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
ROS	Reactive oxygen species
Rpm	Revolutions per minute
SOD	Superoxide dismutase
siRNA	Small interfering ribonucleic acid
snRNA	Small nuclear ribonucleic acid
TAE	Tris acetate-EDTA
TCA	Tricarboxylic acid cycle
TGS	Transcriptional gene silencing
TFs	Transcription factors
tRNA	Transfer ribonucleic acid
UTP	Uridinetriphosphate
μl	Microliter

1. INTRODUCTION

1.1. TOMATO (*Solanum Lycopersicum*)

Tomato (*Solanum Lycopersicum*) is an essential source of antioxidants, which belong to the family of *Solanaceae*, and is necessary for human nutrition. It is a plant species belonging to unique genetic transformation methodology that can be studied [1]. Tomatoes are considered one of the most important food products of nutrition because they are low in fat and do not contain harmful cholesterol. Tomato contains vital compounds such as lycopene, phenolics, and vitamins A, C, and B. Lycopene in its structure are a phytochemical that protects cells from oxidative damage due to cancer[2]. In addition to vitamins, ascorbic acid and potassium are found in significant concentrations in tomatoes. Depending on whether the tomato is raw, ripe, or cooked, the ratio of nutrients in its content varies. The fact that the tomato is ripe or cooked indicates increased phytochemicals concentration. For example, carotene is higher in ripe tomatoes but not in cooked tomatoes. Some studies have found that tomato phytochemicals have a significant effect on preventing respiratory disorders and cardiovascular diseases [3,4]. Ripe tomato fruit is consumed both fresh and used to produce canned products such as canned tomato paste. Unripe fruits are used for making pickles [2].

Tomatoes are mainly grown in the open field, and their production is seasonal. Due to this situation, off-season tomato production in West African regions allows farmers to make profits because of the expensiveness of the market [5]. Along with the development of the social economy and improved living conditions, the demand for tomatoes has been ever-increasing [6]. World Food Organization data, tomato production, has increased proportion to the population from 1994 to 2020. Tomato, a tropical plant, can grow by adapting to almost all climatic conditions [1]. Tomato has been a pattern plant in molecular studies to increase fruit quality and improve resistance to stress conditions [7]. According to FAO world tomato production report in 2020, Türkiye ranked 3rd globally with approximately 13 million 204 thousand tons. (Figure 1.2). In 2020, 5,051,983 tomatoes were harvested, and 186,821,216 tomatoes were produced worldwide (Figure 1.3).



Figure 1.1. Tomatoes Saidin Jusoh/Fotolia

Water is the most crucial factor affecting the yield and quality of tomatoes. A lack of water below a certain degree reduces the crop of tomatoes [6]. When we look at the world in general, it is vital to investigate the drought stress in plants that consume a lot of water, such as tomatoes, due to the gradual decrease in water resources [8].

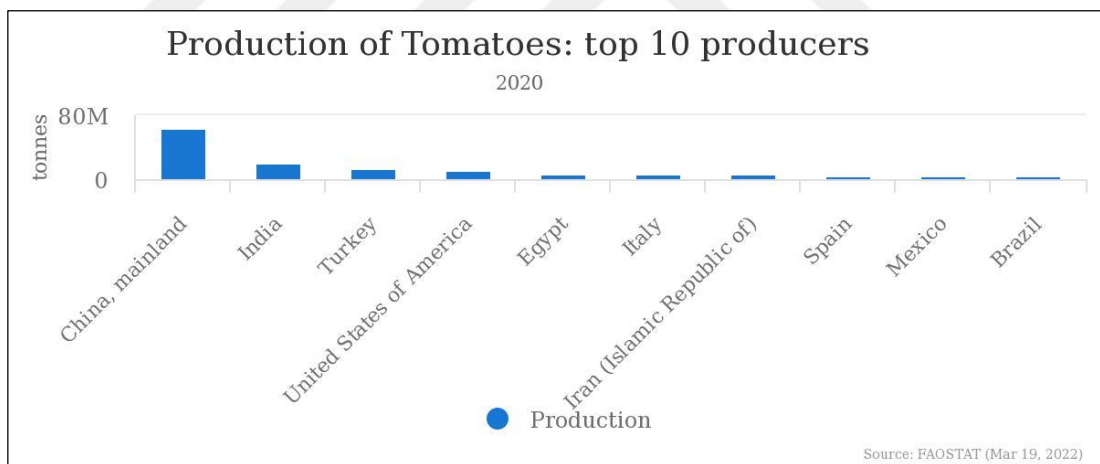


Figure 1.2. Production of tomatoes top 10 Countries in 2020 (FAOSTAT)

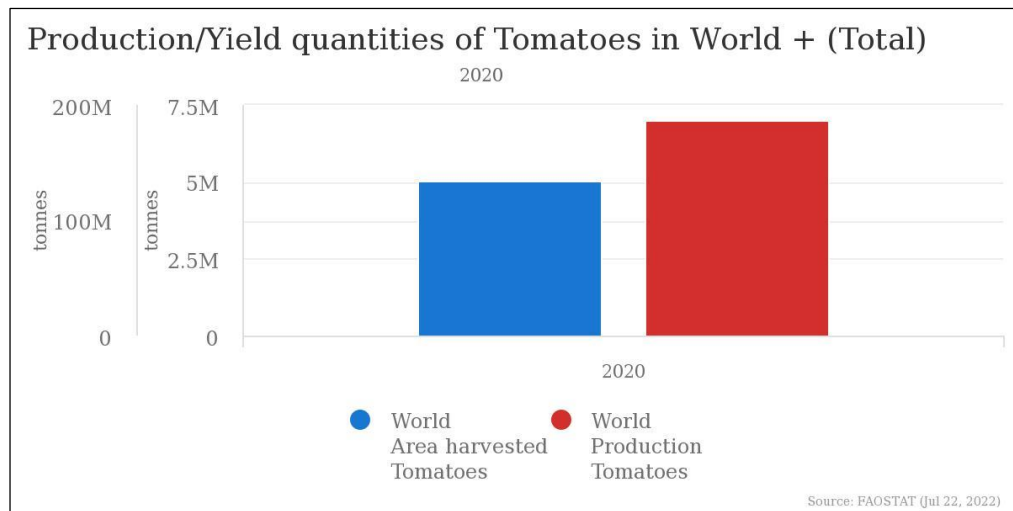


Figure 1.3. Production/Yield of tomatoes quantities in the world in 2020 (FAOSTAT)

1.2. ABIOTIC STRESS

Plants undergo abiotic and biotic stress during their growth [9]. Biotic stress is generated by infection caused by microorganisms (bacteria, fungi and viruses) and the invasion of harmful animals. Abiotic factors, drought, salinity, low and high temperatures, heavy metals and other environmental factors have an adverse impact on the development of plants [10].

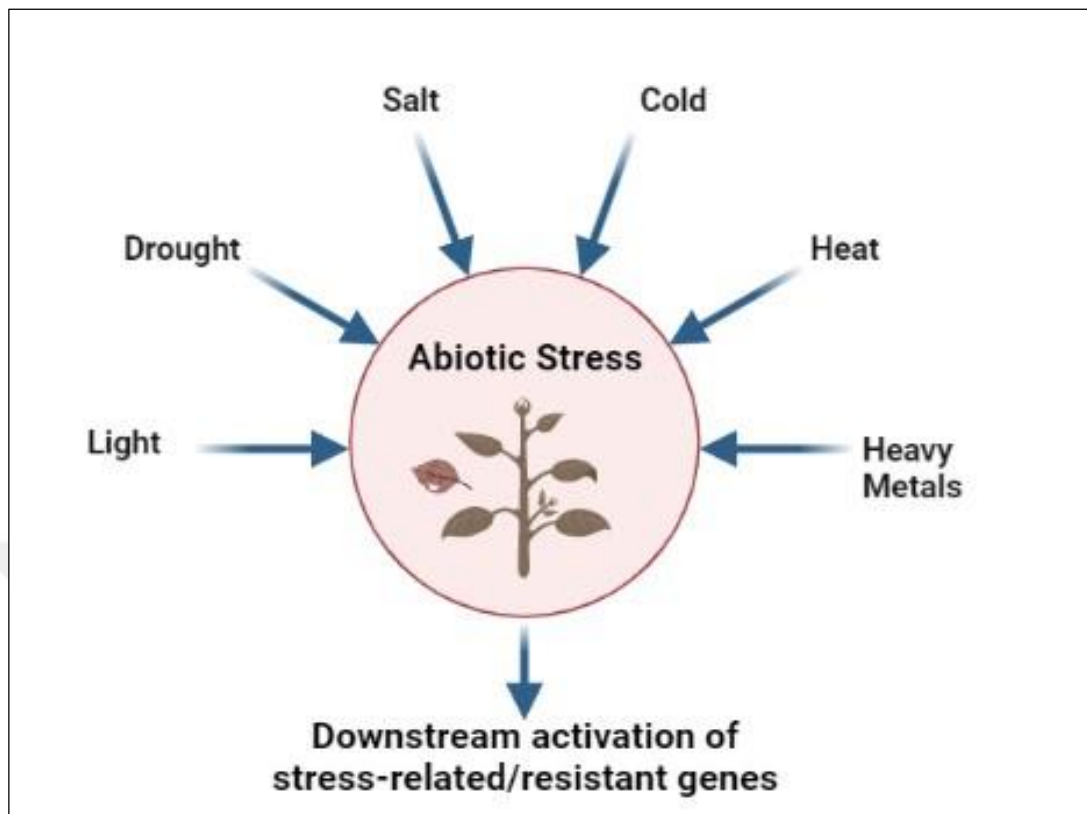


Figure 1.4. Types of Abiotic stress

Excessive dependence on fossil fuels and deforestation are known to increase atmospheric CO₂ pressure [11]. Recently, many countries have become seriously threatened by drought as a result of global climate change. The overuse of water resources has become a global issue. Drought is the abiotic stress that causes the most losses of all [11].

Salinity, which is one of the abiotic stresses, prevents the root part of the plant from absorbing water from the soil. The plant's growth decreases due to the root part not getting the low potential water. The presence of high amounts of Na and Cl ions is toxic to plants and prevents the uptake of minerals by affecting the ion selectivity of the membrane [13]. Heat stress has become a more common problem with increased atmospheric CO₂. High temperature, which is temporary or permanent, negatively affects the development and growth of the plant and causes a decrease in production. In particular, the increase in ion conductivity in the plant causes the deterioration of the membrane structure [14]. About 40% of the land in the world has temperatures below -20°C. [15]. When the plant is under low temperature, the water in the cell freezes, and the cell wall expands and breaks down. In this case, it means the death of the plant [16]. The fluency of the unsaturated fatty acids in the

membrane changes at low temperatures in plants [17]. Another type of stress is light. Light is an essential factor of photosynthesis. However, the intensity above the light required for photosynthesis damages the plant and decreases the rate of photosynthesis [14].

These abiotic stresses that occur in nature do not happen alone, and many stress act with each other. Drought and salt stress disrupt the ionic balance of the cell and affect the function of its molecular parts. Cold stress causes the breakdown of the membrane structure and inhibits the rate of photosynthesis. These effects damage the physical and metabolic processes of the plant. Since abiotic stress responses in the plant cause osmotic balance, there is a connection between drought, salinity, cold stress, and ABA in the expression of stress genes [17,18].

1.2.1. Drought Stress' Physiological Effects

When plants encounter drought stress, their initial response consists of changes in their external structures and internal structures. Slowing in plant growth is the significant impact caused by water deficiency. Especially insufficient water during the vegetative period will cause effects such as fading of the leaf color, decrease in the number of leaves and reduction of the leaf area [19]. Drought is closely related to cell growth, and drought severely affects plant height. Plant height decreases when the plant is exposed to water deficiency and the number of cells decreases and leaves shed. Therefore, leaves, which are the main organs of transpiration and photosynthesis, indicate the degree of water scarcity as a result of drought. Previous investigations have shown that changes in the leaf surface are caused by changes in turgor pressure [20]. In studies on drought, the integrity of the membrane and its effect under stress were investigated while determining resistance. In wheat, cysteine is expressed in leaves and affects leaf development [21]. Another morphological feature that occurs in leave under drought is rolling. This is due to potential internal water failure from the upper epidermis of the leaf [19]. In a way, this is a strategy to avoid drought. Because when the leaf area decreases, water loss by transpiration will be less [22].

In addition to plant roots, directly absorbing water leaves have an essential role in drought stress. Roots that have matured are capable of fully absorbing the water stored in the soil , ensuring the survival of the plant [23]. Fine roots can elongate and thin as a consequence of drought stress [19]. Some research stated that changes such as deterioration in the integrity

of the cell wall, production of toxic metals, and signal transmission of roots occur in water deficiency [21].

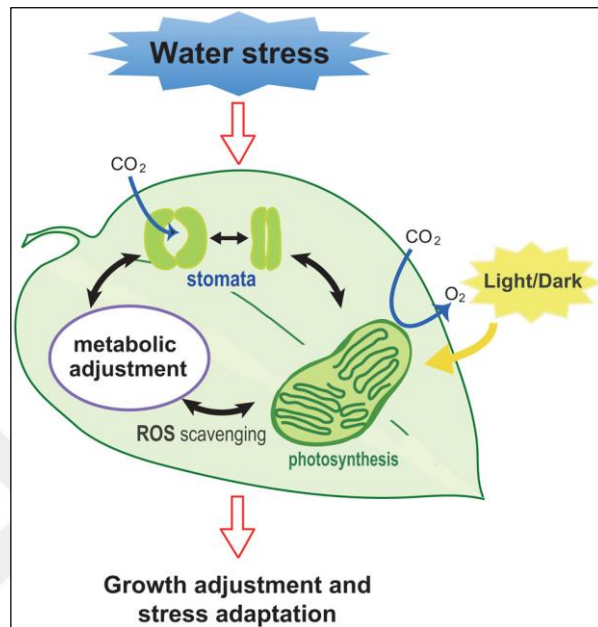


Figure 1.5. The response of the plant to the lack of water[24]

1.2.2. Molecular and Biochemical Effects of Drought Stress

Plants are divulged to diverse environmental stresses while growing under natural conditions. One of the most important of these types of stress is drought stress and it has a negative impact on plant development. Approximately 90% of the plant body consists of water, which ensures the growth and development of the plant. In some cases, even if there is enough moisture in the soil, the root of the plant cannot absorb it, and in this case, pseudo-drought occurs [25]. The physiological, molecular and biochemical properties of the plant deteriorate under drought stress. Genomic approaches have uncovered the genetic mechanism of plants' drought response, which has been made possible by the rapid advancement of molecular biology technology [26].

When a plant is revealed to drought stress, its water uptake and transpiration rate decrease, and the accumulation of some osmolytes [27]. Photosynthetic efficiency in plants is not only dependent on the plant's potential to absorb light energy and produce carbohydrates, but also

on drought stress [28]. It is widely known that drought stress reduces the rate of photosynthesis per leaf area as well as each leaf area. Decreased photosynthesis occurs due to the closure of stomata and metabolic deterioration [29]. It has been determined that stomatal conductivity is liable for lowering the photosynthetic rate under drought stress in soybeans. Photosynthetic pigments are broken down, and plant growth is declined due to reduced gas exchange [30]. Closure of stomata occurs when the turgor pressure of the two guard cells surrounding the stomatal orifice is jeopardized and the orifice closes. There are several signals that cause the stomata to close, the most well-known of which is ABA. [30,31]. The closure of the stomata reduces the intake of carbon dioxide from the environment.

Plant quality and yield are affected by the water deficiency environment. The mechanism of drought resistance also differs among plant species. Networks at the molecular level are responsible for increasing the response to stress, which is involved in signal transmission and when the plant cannot receive enough water. Abscicic acid (ABA) plays a vital role in signalling the opening and closing of stomata under drought stress [24,25]. It increases the conductivity of water and destroys chlorophyll [32]. Droughts impact plants' metabolic function and increase secondary metabolites that protect against water deficiency [33]. Ca^{+2} , ROS, phosphoglycerol, and diacylglycerol play secondary messengers in various signal transmissions [34].

The number of soluble proteins increases in plants not tolerant to drought stress. However, in plants with sensitive genotypes, soluble protein decreases with increasing drought. Soluble protein is liable for maintaining the structure of the plant, and the reduction in soluble protein alterations the structure of the plant [32].

Water, fats, prolines, vitamins, and carbohydrates are components accountable for plant development. Since these components are interconnected, the deficiency in one of them affects the development mechanism of the plant. The balance in the proline and water mechanism is pivotal for the balanced functioning of the plants. In case of insufficient water, it increases proline synthesis for plant growth. Drought stress also boosts the activity of an enzyme called aminotransferase, which is responsible for proline synthesis in plants [35].

The increase in proline in the cytoplasm denotes that the plant faces drought stress. Protein, which is equally essential for the cell in plants, is produced by proline. [36]. Plant cell

constructions are protected by proline, responsible for protein synthesis, so it is considered the plant's defense mechanism against stress. If there is not enough water, sugar synthesis in plant cells also increases. Soluble sugars are components of osmolytes that act as osmotic protection. As the moisture content in the soil decreases, the rate of sugar dissolved in the plant cell increases [35].

Under drought stress, the carbon dioxide balance in the plant deteriorates, increasing the conductivity of the cuticle. RuBP and ATP contents decrease, and Rubisco activity decreases. Stomata close as the carbon dioxide concentration drops [37].

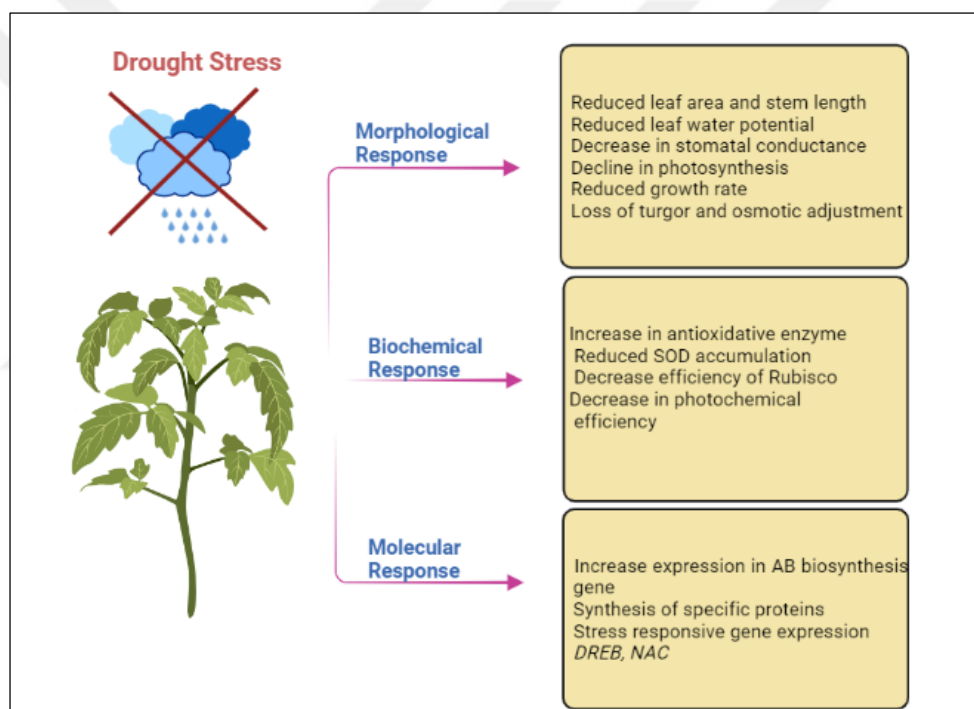


Figure 1.6. Plants Responses to Drought Stress [38]

Drought stress impacts the electron transport chain, which initiates ROS production, harmful to cell organelles like mitochondria and chloroplasts. ROS reduces the amount of chlorophyll in the leaves and decomposes the chlorophyll in the leaves, causing a decrease in photosynthetic pigmentation. In addition, the increase in drought and the decrease in N_2 fixation are associated with raised oxygen resistance caused by ROS [33,39,40]. Most of the total protein and lipid composition of leaf tissue is found in the chloroplasts. Therefore, lipids, one of the main components of the membrane, are affected by drought [41].

1.2.3. Adaptation Mechanisms Developed Against Drought Stress

Two basic defense mechanisms that ensure the plant's survival are insufficient water and development against drought in vegetative tissues. These are stress avoidance and stress tolerance [42].

Responses to water deficiency change according to the plant's genotype, its species, the term of water loss, and the development status of the plant. Succulent plants can be given as an example of a drought avoidance mechanism. These plants can maintain their vitality by storing water in their tissues for a long time. Stress avoidant plants survive moderate water deficits, but stress-tolerant plants can survive under higher drought by activating their protective mechanisms [43,44]. The fact that the upper surface of the leaves of some plants that are resistant to drought stress is covered with a waxy structure is the mechanism developed by the plant to avoid stress. Desert evergreen plants can be given as an example plant for drought tolerance. This plant synthesizes protective solutes to maintain turgor pressure during water deficiency. The changes that occur in the roots under drought stress are for stronger absorption in the soil [42].

One of the first adaptation mechanisms that emerge in the plant that encounters water stress is the closure of stomata to minimize water loss. Because the stomata are open causes the plant to lose more water by transpiration. Therefore, under drought stress, photosynthesis is low [44].

1.2.4. Effects of Drought Stress on *Solanum Lycopersicum*

Although the tomato is a perennial plant that is very sensitive to frost and elevated temperature, it is grown in various climatic conditions. Lack of water in nature is a limiting factor that affects plant development. As water loss progresses, all functions of the plant are negatively affected and cause the plant to perish. An in-depth analysis of environmental and cultural factors aids in the detection of issues. A study in Arabidopsis and tomato determined that the expression of histone-binding genes was induced by drought stress and ABA [45].

Studies have been conducted to show that drought stress adversely affects the reproductive development of tomato plants, especially the male organ. Lamin-samu et al. showed a

reduction attributed to male sterility at different stages of pollen development following flower bud under drought [46]. Deficiency of water in tomato negatively affects cell division and fruit tissue development. It increases ethylene synthesis during fruit ripening and causes an increase in organic acids [8]. In a research published in 2003 by Leperen et al., the hydraulic change of xylem in the fruit bunch in tomato affected by water stress was examined. Xylem and hydraulic resistance were measured directly in this research. The study revealed that the highest resistance was measured in the abscission area (the area where cells secreting enzymes are located). Although plants grown with less water in the root zone have xylem with higher hydraulic resistance in the peduncle and pedicel segments on either side of the abscission area, a tremendous growth in hydraulic resistance was estimated in the abscission area [47].

In their research, Luu and Maurel purported that the water balance in plants should be adjusted not only to harsh environmental circumstances like salinity and drought, but also to changes in nutrient deficiencies and soil acidity. Aquaporin gene transcription and protein abundance can be controlled by these processes. This includes stimulus-induced aquaporin intracellular displacement and channel crossing by reversible phosphorylation [48].

Changes in proline content in tomatoes were detected by physiological analyses in 2006. The impact of different water levels on leaf water potential, stomatal resistance, chlorophyll and protein content, and some enzymes were determined [49]. A similar tomato study revealed that the detrimental effect of drought on chlorophyll and enzymes could be minimized with boron application [27].

Chen applied water stress to plants at different rates from the seedling stage to the fruit stage. This work indicated no substantial impact in the flowering stage, but the fruit quality decreased during the ripening period during fruit formation [6].

The response of an Italian tomato variety to prolonged water deficiency has been extensively studied by Conti et al. This study showed that the Fragola tomato was resistant to drought stress, while the Pisenello variety manifested a genotype-specific effect. Although the Pisenello species was more sensitive and affected more, the raise in the number of stomata and the significant augmentation in antioxidants indicated that it was a sign of oxidative stress. This study enabled distinguishing tomato varieties exposed to drought and to understand the response mechanisms against drought [50].

An increase in the enzyme activities of superoxide dismutase (SOD), Ascorbate peroxidase (APX), and catalase (CAT) applied to four tomato genotypes under drought stress was noticed, and this rise was found to be associated with a protective role against reactive oxygen species (ROS). Protein numbers in drought tolerant and tolerant genotypes were determined to reveal the regulated protein network. Studies in the proteome field have opened the door to comprehending the many metabolic pathways implicated in stress tolerance [1].

In a study conducted in 2022, the defensive function of melatonin (ME) in photosynthesis, root structure, and antioxidant enzyme mechanism in tomatoes under drought stress was investigated. According to the study, it was observed that drought suppressed biomass growth, inhibited photosynthesis and decreased photosynthetic pigments [51].

1.3. NON-CODING RNAS

Protein-coding genes associated with particular target agricultural traits such as nutrition metabolism or pesticide resistance have been identified as the primary goal for creating trait-developed crop plants since the beginning of molecular breeding. Plenty of agricultural features are governed by a complicated regulatory system involving numerous genes; there are primary constraints to changing an agricultural feature by manipulating only a gene [52,53].

Furthermore, crop plants cultivated in fields are frequently exposed to unanticipated stress, which may disrupt the expression of the targeted feature in the area. To circumvent these limitations, several studies have been conducted searching for regulatory genes, like transcription factors, that can control a variety of regulatory networks that affect multiple features. Regulatory non-coding RNAs are becoming a popular goal for genetic engineering [54].

Non-coding RNAs (ncRNAs) are transcripts that do not create convenient peptides or proteins but rather function at the RNA transcript grade rather than as a protein. This includes not just structural ncRNAs (tRNA, rRNA, snRNA, snoRNA, and so on) but also "regulatory ncRNAs. [55]. These regulatory ncRNAs are made up of a variety of small RNAs and long non-coding RNAs (lncRNAs), and they influence target gene expression

through a variety of molecular processes [56]. Recent study on regulating ncRNAs in plants has indicated that they mostly function as master regulators. They regulate a specific group of down-stream genes directly associated to agricultural properties like seed maturity, floral development, disease resistance, and other abiotic stress tolerance, and thus modulate a wide range of gene regulatory networks [57–60].

Some regulatory ncRNAs govern only the target gene, whereas others modulate many genes across the genome through distinct molecular methods. These findings show that regulatory noncoding RNAs may be useful targets for molecular crop breeding. Due to these potential outcomes, many molecular breeders are searching for regulatory ncRNAs connected with agricultural traits that could be candidates for molecular crop plant breeding [54]. Regulatory ncRNAs, including as microRNAs (miRNAs) and small interfering RNAs (siRNAs), are a diverse group of plant short RNAs that mostly function as suppressors in regulatory networks. [61].

Housekeeping ncRNAs include ribosomal (rRNA), transfer (tRNA), small nuclear (snRNA), and small nucleolar RNAs (snoRNAs). Noncoding regulatory RNAs are divided into short ncRNAs (200 nucleotides) and long ncRNAs (>200 nucleotides) and include microRNAs (miRNAs), small interfering RNAs (siRNAs), Piwi-associated RNAs (piRNAs), antisense RNAs (asRNAs), and enhancer RNAs (eRNAs). (Figure 1.7) [61]

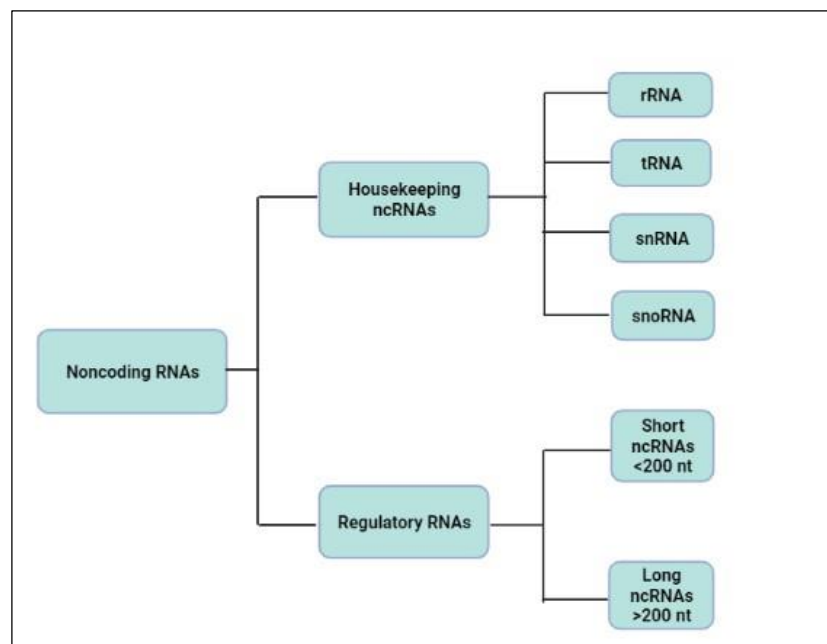


Figure 1.7. Types of noncoding RNAs (ncRNAs) [61]

Recent advancements in sequencing technology have revealed that the noncoding region of the genome plays a significant assignment in transcription factors. Different forms of noncoding RNAs (ncRNAs) various sizes and shapes have been identified in the last three decades, rendering this land-marking idea incomplete to some extent. Different biological and physiological processes have been linked to these ncRNAs. Based ncRNAs size can be separated into three families. Short ncRNAs (RNAs with lengths between 17 and 30 nt), middle-size ncRNAs (RNAs with lengths between 30 and 200 nt), and long ncRNAs (RNAs with lengths greater than 200 nt) [62].

Plant ncRNAs are split into two classes based on their molecular form: linear ncRNAs and circular ncRNAs. In comparison to circRNAs, which were just discovered as a new class of ncRNAs, the most well-known ncRNAs are linear ncRNAs. Linear ncRNAs can be split into two categories based on their molecular processes: (1) housekeeping ncRNAs, which include rRNAs, tRNAs, and snoRNAs; and (2) regulatory ncRNAs, which are further divided into two sub-categories: sRNAs, such as miRNAs and siRNAs, and lncRNAs, such as long intronic ncRNAs and long intergenic ncRNAs, are two types of small RNAs. The circRNAs can be categorized into five classes based on the genome area in which they are found: (1) exonic circRNAs, (2) intronic circRNAs, (3) UTR circRNAs, (4) intergenic circRNAs, and (5) additional circRNAs derived from two or more genes [63,64].

The study of the functions of ncRNAs has concentrated on sRNAs, lncRNAs, and circRNAs, which have been found to be important in the control of plant growth, development, and stress response in a variety of plant species. sRNAs have been shown to regulate plant growth, development, and stress response by inhibiting endogenous gene expression at either the transcriptional or post-transcriptional levels, according to research. miRNAs are divided into two types: conserved miRNAs and non-conserved miRNAs. Conserved miRNAs are abundantly produced and directly regulate gene expression by targeting transcription factors. As a result, studies show that the regulatory efficacy of conserved miRNAs over their conserved targets might vary depending on precursor processing efficiency as well as minor changes in the mature miRNA sequence. Non-conserved miRNAs, on the other hand, are poorly described and have been displayed to occur in temporal patterns. Therefore, they are thought to have developed at random with a restricted number of biological functions. According to recent research, miRNAs have a part in plant growth and improvement, including organ morphogenesis, hormone signalling, and reactions to diverse external

environmental stimuli [59,63,65].

siRNA are classified siRNAs are classified into three groups: (1) heterochromatic siRNAs (hc-siRNAs), (2) secondary siRNAs, and (3) natural antisense transcript siRNAs (NAT-siRNAs), all of which are produced via Dicer-like (DCL)-catalyzed processing of double-stranded RNA (dsRNA) precursors. Although miRNAs are implicated in PTGS via transcript cleavage or translational repression, and may induce secondary siRNA synthesis from Pol II-derived cleaved transcripts, the bulk of siRNAs in plants are united with RNA directed DNA methylation (RdDM) and transcriptional gene silencing (TGS) [63,66].

Variant splicing event known as back-splicing has been widely documented to be the source of circRNAs in mammals and plants. Circular RNAs have covalently shut continuous circles with no 5' to 3' polarity and poly (A) tails, unlike linear RNAs [67]. Plant circRNAs differ from animal circRNAs in a number of ways. The bordering introns of circRNAs in animals, for example, are enriched in reverse complementary elements, which are necessary for circularization. On the other hand, most of the discovered circRNAs have fewer recurrent and reverse supplementary sequences in the adjacent introns that surround the circRNAs. Plant circRNAs were originally identified genome-wide in *Oryza sativa* and *Arabidopsis thaliana*. 12,037 circRNAs were found in the roots and shoots of *O. sativa*, and 6,012 circRNAs were found in the leaves of *A. thaliana* [68].

1.4. LONG NON-CODING RNAs

The existence of long noncoding RNA in plants has been acknowledged for many years, but its role and mode of activity are still not comprehensively known. By this time, thousands of new lncRNAs have been discovered in many plant species through experimental studies. With the development of RNA-seq applications, lncRNAs are studied and identified at a specific genome scale. Long noncoding RNAs (lncRNAs) have a nucleotide length of more than 200 base pairs and an open reading frame of less than 100 amino acids [69].

lncRNAs can be polyadenylated and nonpolyadenylated. Nonpolyadenylated lncRNAs are shorter than polyadenylated lncRNAs, with 50–300 nucleotides in length and limited expression, but they respond more specifically to stress than polyadenylated lncRNAs. RNA polymerase III is also required for transcription of nonpolyadenylated lncRNAs (poly(A))

[70–72]. Adenosines are added to the 3 ends of eukaryotic messenger RNA to form poly A tails. Transcriptional cleavage of these RNAs occurs at a poly(A) site in the nucleus, followed by polyadenylation and export to the cytoplasm. Poly(A) tails are essential for the stability and translation of mRNA in the cytoplasm [73].

Long non-coding RNAs can have very similar properties to mRNAs. Therefore, it should investigate whether lncRNAs have the possibility of coding proteins. Ribosome profiling enables translational analysis. This method provides translational research that involves the digestion of RNAs that are not bound by ribosomes [63]. RNA fragments are bound by ribosomes and protected from RNases. Then, RNA Fragments are precipitated by sucrose gradient sedimentation due to size. The ribosomes are then deconstructed, and the remaining RNA fragments are sequenced. Transcripts are formed in different sizes depending on the fragmentation state, and short or repetitive sequences occur [63].

Many studies have been done on lncRNAs in animals, but lncRNA studies in plants are limited. According to the research, found that lncRNAs are involved in stress response and plant ripening and development. For instance, Photomorphogenesis, auxin-controlled growth, reproductive processes, root genesis, biotic and abiotic stress responses, and so on are all regulated by lncRNAs. [74]. New long non-coding RNAs can be determined in two ways, either through experimental or bioinformatics approaches. More than 200 transcriptional data sets from *Arabidopsis thaliana* from organ-specific or stress-induced tissues have been studied to date and approximately 6480 lncRNAs have been determined [65]. Likewise, studies on the flowering of *Arabidopsis* under stress conditions could detect the presence of lncRNAs without poly-A tails. However, it was determined that the lncRNAs that did not carry this poly-A tail were shorter than the ones carrying the poly A tail and their expressions were very low and they were original in the response to stress [71]. Both bioinformatics and experimental analyses have shown that 125 lncRNA, which are thought to be effective in response to stress in wheat, are induced in temperature stress together with tissue specific and powdery mildew disease [75].

Furthermore, 30 different maize genomes have been reported in recent studies where 20,163 possible lncRNAs were identified by combining EST and RNA sequencing data. It is thought that more than 90% of the obtained corn lncRNAs are tissue-specific and potential reporters of sRNAs [71]. In a paper in 2014, Zhang and colleagues were able to identify 2224 lncRNA

by a combination of previously obtained RNA-seq data of the sequence-specific RNA sequencing results of rice anther, pistil, seed and seedling [76].

In addition to Arabidopsis, rice, and maize, lncRNAs have been recognized in soya, where many endogenous target mimics (eTMs) occur. It has been determined that these lncRNAs are complementary to miRNAs. Instead of attaching themselves to the gene that organizes the essential expression of the miRNAs, they attach themselves to the lncRNAs and acting a role in the organization of the gene expression [77].

In a study by Ye et al. (2014) it was demonstrated that through some eTMs and as a result of the organization of miRNAs expression, the expressions of genes associated with lipid metabolism had changed. Similar studies for the identification of more recent lncRNAs have been carried out with the use of RNA sequencing strategies for peaches poplars and *Brassicarapa* [64,78].

lncRNAs play a vital role in the ripening of some plants' fruits. During tomato fruit ripening, 3679 lncRNAs were found in previous investigations, with 490 lncRNAs showing high expression and 187 lncRNAs showing low expression [79].

1.4.1. Classification of Long Non-Coding RNAs

lncRNAs are mainly classified according to four main conditions, namely genomic locations, their functions on DNA or RNA, functional mechanisms and being targeted / targeting mechanisms [80].

A more precise classification to describe the diversity of lncRNAs has been proposed in a recent study. lncRNAs are divided into seven classes in this new classification. 1) anti-sense transcripts of protein coding genes (natural anti-sense transcripts -NATs-), 2) promoter upstream transcripts (PROMPTs), 3) mRNA-like intergenic transcripts (lincRNAs), 4) processed transcripts, 5) enhancer RNAs (eRNAs), 6) circular intronic RNAs (ciRNAs), and 7) lncRNAs with snoRNA (small nucleolar RNA) ends (sno-lncRNAs) [70].

Most Pol II-transcribed lncRNAs have a 5' cap and a non-adenylated or polyadenylated 3' tail. Like Pol IV and Pol V, other RNA polymerases are crucial in synthesizing lncRNAs. Compared to lncRNAs produced from Pol II, which have poly-A tails and play a significant

role in promoting RdDM (RNA-directed DNA methylation), lncRNAs transcribed from Pol IV and Pol V lack poly-A tails and are less expressed. According to the biogenesis loci, lncRNAs are divided into intergenic, sense or overlapping, intronic, antisense, and bidirectional. [81,82].

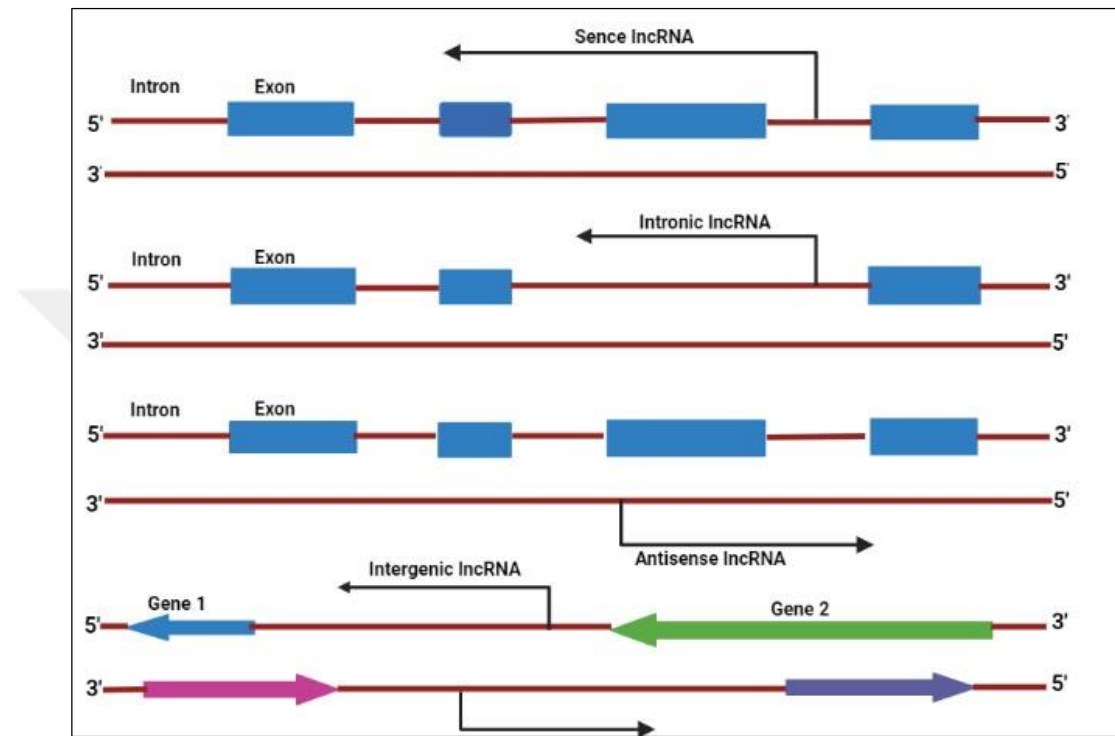


Figure 1.8. The position of lncRNAs in relation to protein-coding genes is used to classify them

lncRNAs copied from the same region as a protein-coding gene with a gene that disseminates the identical promoter is called sense lncRNA. Those originating from the intron of the gene encoding the protein are called intronic lncRNA. The best instance of sense and intronic lncRNA studied in plants is COLD AIR (COLD Assisted Intronic noncoding RNA). This lncRNA regulates the silencing of the flower suppressor FLC in the plant. lncRNAs in the antisense direction (reverse strand) is called antisense lncRNAs. They are shown either in concordance with the sense strand or in a discordance method [83]. In a study in rice plants, cis-acting antisense lncRNA is transcribed from the contrasting sequence of the R2R3 MYBP gene locus, named OsMYB60. This antisense lncRNA is related to the growth of leaf structure by suppressing the sense genes in rice [84]. Intronic transcripts with

no exon overlap are known as sense intronic lncRNAs. They are found inside the introns of coding genes [85].

1.4.2. Long Non-Coding RNAs Functions

lncRNAs regulate gene expression through various mechanisms at the transcriptional and post-transcriptional stages. They interact with chromatin regulatory proteins, they act as miRNA bait and epigenetic silencing [81]. lncRNAs have been shown to play functions in responses to infection in addition to sexual reproduction, tissue development, drought, and excessive temperature changes in recent years [75]. The roles of short coding non-coding RNAs including siRNAs and miRNAs in transcriptional and post-transcriptional regulation of gene expression have been described and the regulation mechanism of short non-coding RNA-mediated gene expression has been understood while the molecular basis of the gene expression regulation of lncRNAs has still not been fully demonstrated.

Because lncRNAs may interact with nucleic acids (DNA, RNA), they play a role in molecular methods, including epigenetic and (post)-transcriptional alterations, translational control, splicing, and scaffolding. lncRNA's various functions are associated with the cellular location. lncRNA transcripts are mostly present in the nucleus and cytoplasm, however some can also be detected in exosomes. Nuclear lncRNAs are related to chromatin structures and control gene expression through a variety of methods, including transcriptional and epigenetic regulation of particular genes [70,86].

Over the last few years, research into the role of lncRNAs in plant gene regulation has increased dramatically, highlighting the similar methods utilized to regulate transcription across eukaryotic genomes as well as their complexity. lncRNAs change DNA methylation, DNA structural changes, histone modifications, and RNA-RNA interactions in order to function. Despite the fact that these regulatory RNAs are categorized together as lncRNAs, they are produced by separate processes and constitute a cross-network of RNA metabolism that is mutually connected [87,88].

It has been found that lncRNAs have a role in the development process of the plant, as well as its relationship with the stress mechanism. They are a crucial component of the transcriptome involved in the regulation of different biological processes. These biological

functions are nodule development, productivity, auxin regulation, fiber development, vernalization, and fertilization. [69,89]. Photomorphogenesis is an essential environmental factor in light-dependent plants. The regulatory factor involved in this pathway in



Table 1.1. List of biological functions and modes of action of long non-coding RNAs [90]

SPECIES	Name	Mechanism of action	Biological function
<i>Arabidopsis thaliana</i> (<i>Arabidopsis</i>)	IPS1	Target mimicry: interacts with an miRNA, ath-mir399	Regulates phosphate homeostasis
	COLD AIR	Epigenetic silencing: modification of histones	Regulates flowering
	COOL AIR	Epigenetic silencing: Promoter interference	Regulates flowering
	HID1	Stage specific: Associates with chromatin and represses transcription of phytohormone interaction factor 3 (PIF3)	Regulates photomorphogenesis
	asHSFB2a	Hijacks nuclear Antisense transcription regulators	Regulates the gametophytic and vegetative development
	ASCO-lncRNA	Regulators of alternate splicing	Regulates the development of lateral roots
	APOLO	Causes the formation of Chromatin loop and regulates the expression of its neighboring genes	Regulated plant development through auxin signaling
	ELENA1	Increases the expression of genes involved in plant immunity, such as PR1 and PR2, B 1,3glucanase and salicylic acid genes	Upregulated in disease caused by <i>Pseudomonas syringae</i>
	Drought induced RNA (DRIR)	Regulates stress responses such as stomata closure	Upregulated in drought and salt stress
	CDF5 LONG NONCODING RNA (FLORE)	Natural anti-sense transcript of CDF5, upregulates flowering	Photomorphogenesis
<i>Glycine max</i> (soybean)	GmENOD40	Involved in the development of nodules	Regulates the re-localization of proteins from nucleus to cytoplasm
<i>Hordeum vulgare</i> (Barley)	HvCesA6 lnc-NAT	Acts as a precursor for siRNA	Involved in the synthesis of cell wall
<i>Medicago truncatula</i> (Barrel medic)	MtENOD40	Involved in the development of nodules	Regulates the re-localization of proteins
<i>Oryza sativa</i> (Rice)	OsPI1	Unknown	Regulates phosphate homeostasis
	OsENOD40	Involved in the development of nodules	Unknown
	LDMAR (P/TMS12-1)	Acts by causing promoter methylation	Male fertility regulation
	Cis-NATPHO1;2	Acts by enhancing translation of <i>PHOSPHATE1;2</i>	Regulates phosphate homeostasis
	lncRNAs: 2224 lincRNAs: 1624 lncNATs: 600	Tissue and stage specific: Anthers, pistils, seeds five days after pollination, and shoots 14 days after germination	Regulates sexual reproduction
<i>Petunia hybrid</i> (Petunia)	SHO lnc-NAT	Degrades dsRNA	Regulates the synthesis of local cytokines
<i>Populus trichocarpa</i>	lncRNA20 lincRNA2752	target mimic of ptc-miR476 target mimic of ptc-miR169	Upregulated in abiotic stress (drought)
<i>Solanum lycopersicum</i> (Tomato)	TPS11	Unknown	Regulates phosphate homeostasis
	Slylnc0195 slylnc1077	target mimic of miR166 target mimic of miR399	Upregulated in infections caused by tomato yellow leaf curl virus

Arabidopsis is PIF3. PIF3 encodes the main helix loop, the transcription factor. In a study in Arabidopsis, lncRNAs that modulate PIF3 expression and play a crucial role in photomorphogenesis were identified [91].

The molecular agents underlying plant male sterility have not been extensively investigated. A study conducted in rice plants investigated the effect of lncRNAs on fertility. A photoperiod-sensitive mutant was found in a survey of rice. The fertility-related RNA (LDMAR) of the mutant found, called Long-Day-Specific Male fertility, was found to be regulated by a 1236 bp lncRNA. [69,92]. Another study showing the relationship between lncRNA and fertility was done in cotton. The lncRNAs found in this study were the pioneering work in improving fertility and improving cytoplasmic male sterility lines in various crop plants. [69].

Vernalization initiates and controls flowering in plants. The Flowering Locus C gene is a critical regulatory gene that starts from the vegetative part and plays a role in the transition to the reproductive stage. FT proteins control the growth of the flower organs in the apical meristem. The FLC gene locus indicates the formation of COOLAIR and COLDAIR. These lncRNAs modulate FLC expression at the transcriptional level through histone modification [93,94]. According to Swiezewski study, COLDAIR and COOLAIR, two Arabidopsis lncRNAs, have been shown to be involved in the chromatin modification-mediated silencing of FLC (FLOWERING LOCUS C) during the vernalization process [95].

Kang and Liu stated in their study that lncRNAs have significant roles in flowering in RNA sequencing of plants such as *Poncirus trifoliata* and *Fragaria vesca*. Identifying various lncRNAs in flower and fruit tissues suggested the effect of lncRNAs on reproductive tissue. [96]. One of the functions of lncRNAs is to include processes directly related to plant productivity. *Brassica campestris* ssp. *chinensis* BcMF11 is explicitly found in pollen and is required for pollen development. Similarly, it is an antisense lncRNA against MpFGMYB, an essential regulator of female sexual tissue differentiation in liverwort [97,98].

In Arabidopsis, auxin-sensitive APOLO is involved in the transcription of the PINOID (PID) gene through the chromatin loop formation. The expression level of APOLO determines the chromatin environment and DNA methylation level of PID, which affects histone modifications. In addition, APOLO regulates R-loop formation and trans target loci mediated by short sequence complementarity [99]. lncRNAs are components of the

telomerase mechanism. For example, lncRNA AtTR is the RNA subunit of telomerase that interacts with TELOMERASE REVERSE TRANSCRIPTASE (TERT) to maintain the integrity and stability of telomeres [97].

In a study on cotton, it has been shown that lncRNAs contribute to fiber development by showing changes in the elongation part [100]. Earlier research has shown the effect of lncRNAs on root development. For example, Auxin-dependent lateral-root development is regulated by Nuclear Speckle RNA binding proteins in Arabidopsis, according to Bardou's research (NSRs). These proteins are controlled by long non-coding RNA [101].

1.4.3. Stress Responsive Mechanisms lncRNAs

Researcher used a variety of ways to identify the important function of lncRNAs in abiotic stress response, which can be applied to design abiotic stress-tolerant cultivars. Some search has shown that lncRNAs play critical roles in some developmental processes in plants [102]. In addition, the findings suggested that lncRNAs involved in several stress responses. A few drought stress-sensitive lncRNAs have been found in plants such as maize, foxtail millet, alfalfa, populus, and others based on expression profiling. LncRNAs that respond to drought were found in 504 and 664 maize and poplar plants, respectively [76]. The favourable influence of DROUGHT-INDUCED lncRNA (DRIR) on Arabidopsis plants during drought and salt stress was demonstrated in an experiment [103]. According to Xuan's study, 1060 stress-related lncRNAs from 43 plant species and 17 diverse abiotic or biotic stress conditions in the PLNlncR base database [104].

In distinct ways, lncRNAs in some plants may have a role in stress response. lncRNAs were expressed in Arabidopsis under different stress types such as drought, salt and cold. In addition, drought-responsive lncRNAs were discovered in maize, including intergenic and antisense sequences. In addition, numerous assumed lncRNAs, intronic, intergenic, sense, and antisense lncRNAs were organized in response to osmotic stress and salinity in the model legume *Medicago truncatula*. All studies show that different types of lncRNAs are related in response to stresses even though lncRNAs' roles, molecular mechanisms, and interactions are unknown [105].

Although lncRNAs have a limited ability to produce proteins, they play an essential role in

controlling the expression of target genes during transcription and translation. Plant lncRNAs are well-known for controlling complex gene regulatory networks involved in plant development and stress tolerance.

Thousands of lncRNAs were discovered in *Arabidopsis thaliana*, *Zea mays*, *Triticum aestivum*, *Oryza sativa*, *Cucumis sativus*, and *Medicago truncatula* using in silico predictions and tiling arrays at the whole genome and RNA sequencing (RNA-Seq) levels. The majority of the lncRNAs that have been discovered are stress-responsive [106].

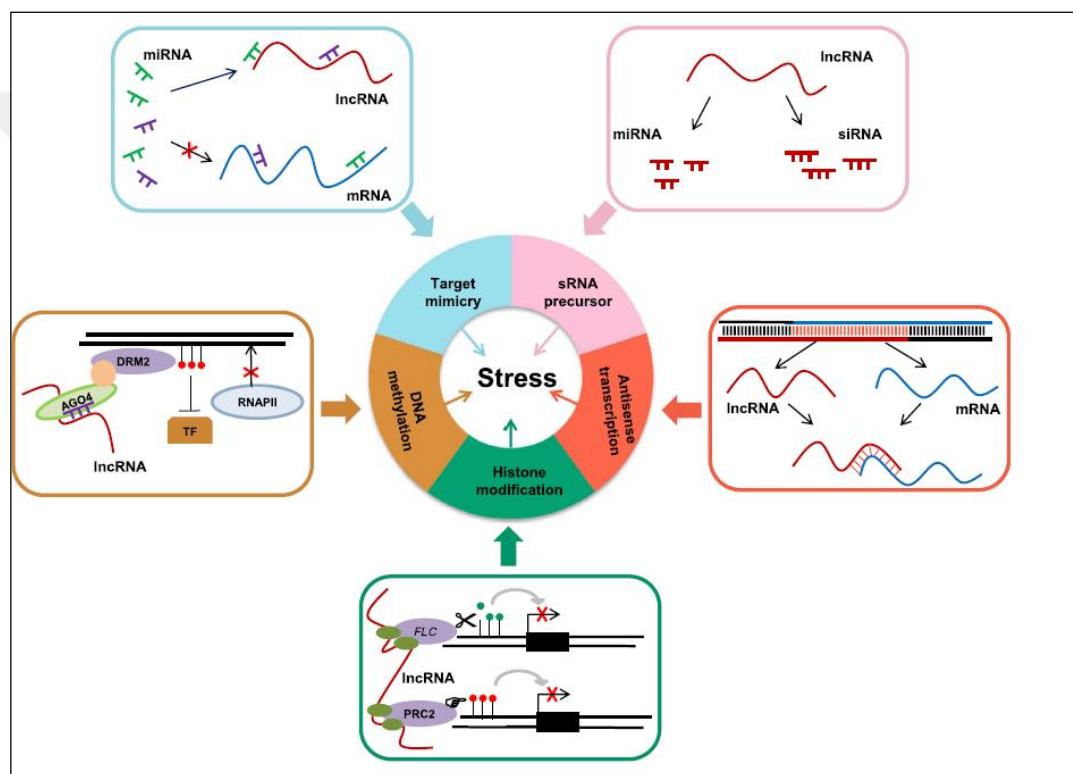


Figure 1.9. Roles of lncRNAs in stress response in plants [107]

lncRNAs can perform stress response functions in plants in five different ways (Figure 1.7). First one, some lncRNAs in plants can act as ceRNAs. Specific miRNAs are targeted by target mimicry, blocking interactions between the miRNA and its targets. Second, some lncRNAs can act as sRNA precursors. Just like miRNAs and si RNAs, sRNAs are produced. Third, antisense lncRNAs respond to stress by interacting with sense mRNAs. Fourth, specific lncRNAs are responsible for controlling chromatin changes (lncR2Epi). Fifth, lncRNAs have a significant role in DNA methylation (RdDM) in the stress response. siRNAs and lncRNAs are involved in the establishment of novo cytosine methylation [107].

Intergenic (2821), antisense (111), exonic overlap (76), and lncRNA isoforms were discovered in a study on *Brassica napus* (173). Furthermore, the originators of miRNA156, miRNA169, and miRNA394 have been identified as 41 lncRNAs. These microRNAs were found to play a significant role in modulating plant responses to fungal infections. In response to *Sclerotinia sclerotiorum*, 931 lncRNAs were identified, and 12 lncRNAs were used to confirm their expression using qRT-PCR, revealing distinct expressions [106,108].

The Illumina sequencing technique was used to sequence susceptible and tolerant banana genotypes to identify differential expression in both control and stressed settings. After rigorous filtering, strand-specific RNA-Seq of *Fusarium oxysporum*-infected susceptible and resistant banana cultivars found 5294 lncRNAs with high confidence, including intergenic and antisense lncRNAs [109]. A differential expression study identified 19 lncRNAs in foxtail millet under osmotic stress brought on by polyethylene glycol (PEG), including 17 lincRNAs and two NATs [78].

Plants detect cold stress by signal transduction, which causes transcription factors and cold-responsive genes to become active. The majority of these are regulated by lncRNAs, which aid in plant tolerance to cold stress. Two lncRNAs that impact the expression of the FLC gene in *Arabidopsis* are COOLAIR and COLDAIR. During chilly temperatures, this gene works as a flowering repressor [69].

Several investigations have suggested that lncRNAs may have a function in bacterial infections. In potato defending responses against the bacteria *Pectobacterium carotovorum* subsp. *brasiliense*, 1113 lincRNAs are implicated [110]. According to Ly study, Maize transposable elements have a function in the production of long non-coding RNA, which serves as a regulatory center for the plant's abiotic stress response [111].

The biological roles of lncRNAs may be experimentally validated in a wide range of ways, providing detailed knowledge about their functional originality in plants. Furthermore, a thorough examination of interaction partners and miRNA targets of lncRNAs might aid in the discovery of stress-responsive gene-regulatory systems [69].

1.4.4. Investigation of Long Non-Coding RNAs on Tomato

lncRNAs have been recognized and researched in various exemplary and produce plants, including *Arabidopsis thaliana*, *Solanum Lycopersicum*, *Oryza sativa*, *Zea Mays*, *Triticum aestivum*, *Glycine max*, *Solanum tuberosum*, and, thanks to advances in sequencing technology. Research on tomatoes lncRNA have shown the significance of lncRNA in regulating tomato fruit ripening and growth. The tomato plant has been the subject of numerous investigations in recent years [112].

In general, tomato lncRNAs have been identified and their association with fruit ripening, coloration, pigment deposition, fruit chill damage, and DNA methylation has been determined. Several lncRNAs have been recognized in tomato plants involved with tomato yellow leaf roll virus (TYLCV). Slylnc0049 and slylnc0761 expression, for example, can prevent TYLCV infection in tomatoes, while inhibiting lncRNA 1459 expression can impede fruit ripening [112–114].

Flowering is an important part of plant development that affects fruit formation. A total of 10,919 lncRNAs, 248 of which were new lncRNAs, were identified during the flowering stage of the tomato plant [115]. These studies show that lncRNAs in tomato plants play significant regulatory roles in the plant's development process, fruit formation process and stress reactions. A research carried out by Ning et al. in tomato plants under salinity stress revealed 1044 lncRNAs and that differentially expressed lncRNAs responded positively to salinity stress by accession in the ABA signalling pathway through the functional analysis of their target genes [116]. 3679 lncRNAs were discovered in a study on wild tomatoes. 85 percent of these lncRNAs are transcribed from intergenic zones on all tomato chromosomes. Tomato lncRNAs are smaller and contain fewer exons than other lncRNAs and protein-coding genes. In the study, 490 lncRNAs were up-regulated, while 187 lncRNAs were down-regulated in ripening fruits. This indicates that lncRNAs have a function in the ripening of fruits [75;112]. Deep sequencing was performed in high-expression LeERF1 tomato fruits to understand the roles of lncRNAs in ethylene signalling in tomatoes. As a result of sequencing, a total of 397 lncRNAs were investigated, 169 of which had not been identified before. Numerous lncRNA target genes have been identified, including oxy-induced proteins, and MADS-box proteins [118].

1.5. AIM OF THE STUDY

Increasing population and decreasing water resources in the world increase the demand for food. Drought, one of the most important types of stress faced by plants, shows its effect on agriculture daily. This situation is affected agricultural products directly. Under drought stress, adverse physiological, biochemical, and molecular effects occur in the plant. In studies conducted years ago, the regulation of gene expression under abiotic stresses was limited to protein-coding genes. However, studies have revealed that non-coding RNAs have regulatory and functional roles under stress. Studies on the identification and function of lncRNAs are limited.

This study aims to analyse the physiological and molecular analyses of two tomato cultivars (Falcon and SC2121) under drought stress and to investigate long non-coding (lncRNAs). The physiological effects of drought stress will be examined by applying shoot and root height, water content and soluble protein content analyses. lncRNAs will be detected by next-generation sequencing method from tomato leaves isolated from RNA as molecular analysis. 3 lncRNAs with high expression will be selected from the determined lncRNAs, and sequencing verification method will be applied by RT-PCR method. In addition, it is aimed to investigate the functions of lncRNAs that express under drought stress.

2. MATERIALS AND METHODS

2.1. ANALYSIS OF THE PHYSIOLOGICAL VARIATIONS

2.1.1. Plant Growth and Drought Treatment

SC2121 and Falcon tomato varieties were used in this study. Tomato seeds were provided from “Arzuman Tohumculuk”. Tomato seeds drill in soil under greenhouse conditions (50-60% humidity and 23-26 °C) for 20 days. Irrigation was carried out by giving a total of 150 ml of water in the morning and evening. After the plants have more than 3-4 leaves, drought stress is applied for seven days. After that, roots and leaves were reaped, and they were stored at -80 °C until RNA isolation [27].

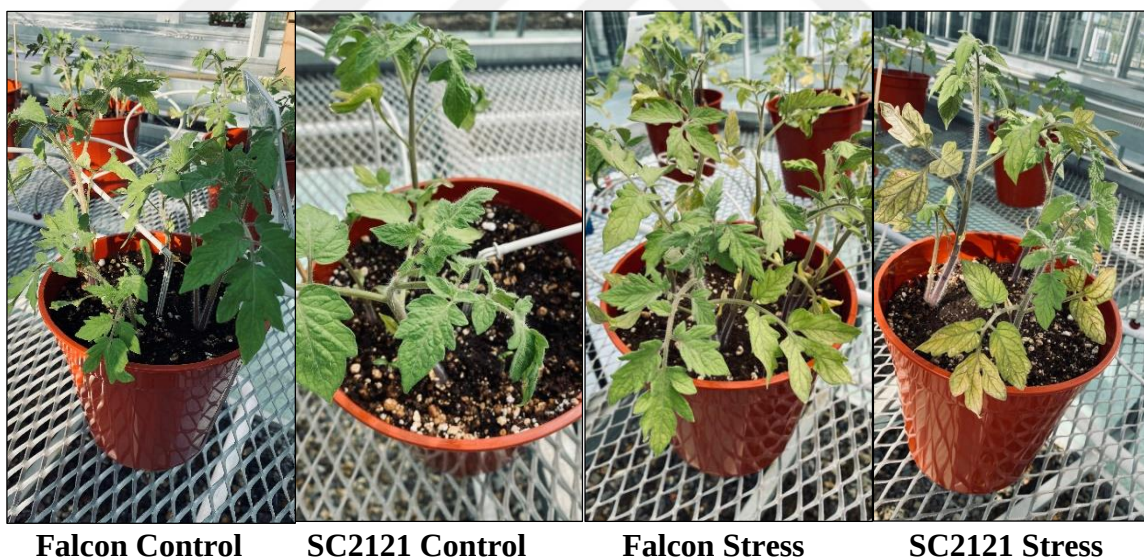


Figure 2.1. (*Solanum lycopersicum*) Tomato plants control group and after 7day drought stress application



Figure 2.2. Tomato samples after the applied drought stress

2.1.2. Chlorophyll Amount

Chlorophyll content of tomato plants subjected to drought stress was measured using the Konika Minolta Chlorophyll meter at the end of the 1st, third, and seventh days. Measurements were taken from four leaves in 3 repetitions.

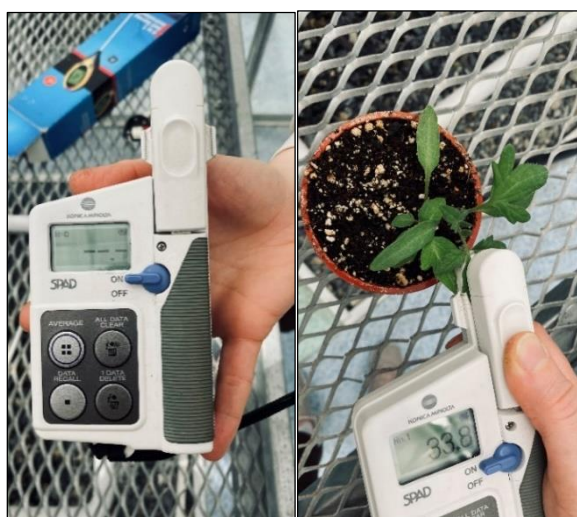


Figure 2.3 Tomato leaf chlorophyll was measured by Konika Minolta Chlorophyll meter (SPAD 502)

2.1.3. Measuring of Soil Moisture

During drought the application of soil moisture was measured daily by the soil survey device, and soil moisture and soil dryness degree were noted.

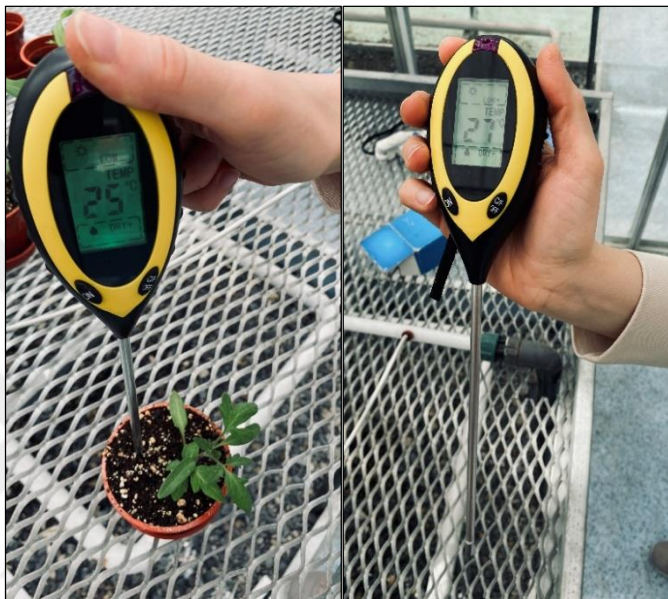


Figure 2.4. A moisture device measured soil moisture (Brand:Generic)

2.1.4. Determination of Root and Shoot Height

After seven days of drought stress, seven replicates of the control and treated plants (seven plants of each) were harvested. The root and shoot of each plant were collected to estimate fresh shoot height. The shoot and root height were measured [63].

2.1.5. Determination of Water Content

Water content (WC %) of plants as control and treated drought was estimated. After 7 days, the plant's fresh leaves weights were measured. Measured the fresh plants were dry in the oven for two days at 65 °C, and after that, the oven-dry plants' weight was measured [63]. WC (%) was calculated by using the $WC (\%) = [(FW - DW) / FW] \times 100$ formula

DW= Dried weight

2.1.6. Determination of Soluble Protein Content

The increase in total soluble proteins in plants under drought is due to the accumulation of small protein molecules due to plants' high amino acid content. In other words, the osmotic balance of the plant is disturbed. Therefore, soluble protein content analysis is one of the most important parameters required for physiological analysis in case of water deficiency [119]. Tomato leaves were crushed in liquid nitrogen. BSA standard solution was created at various concentrations by diluting it with ddH₂O. After that plant sample was mixed with a working reagent and measured absorbance at 562 nm. The soluble protein content was determined according to the (BSA, # 23225, Thermo-Fisher) BCA Protein Assay Kit.

2.1.7. Determination of Lipid Peroxidation Test (MDA)

According to the Wang study, the quantity of malondialdehyde (MDA) measured was used to detect lipid peroxidation (Wang et al., 2013). 0.5 g plant tissue was collected and mixed with a 5% trichloroacetic acid (TCA) solution. For 15 minutes, the homogenate was spun at 5000xg. The supernatant was then collected, and the thiobarbituric acid (TBA) technique was used to calculate MDA levels (Hodges et al., 1999). 3 ml of 0.5 percent TBA, containing 5% TCA, was added to the milliliter extraction solution. After 30 minutes of incubation at 95°C, the mixture was centrifuged at 5000xg for 15 minutes. The MDA concentration was determined at three distinct wavelengths: 600, 532, and 450 nm in the supernatant. The formula was used to determine the MDA concentration. [120] $C_{MDA} (\mu\text{mol ml}^{-1}) = 6.45 \times (D_{532} - D_{600}) - 0.56 \times D_{450}$

2.2. MOLECULAR EXPERIMENTS

2.2.1. RNA Isolation

The manufacturer's recommended procedure was used to extract total RNA from tomato leaves (Trizol Reagent, Invitrogen). Weighing around 100 mg of plant tissue and placing it

in tubes with liquid nitrogen. The frozen tissue was then mixed with 400 μ l of Trizol. The samples were then homogenized and 600 μ l of Trizol were added. The material was vortexed for 15 seconds before incubating for 5 minutes at room temperature. The tubes were filled with 200 μ l of chloroform and shaken for 15 seconds. After that, samples were centrifuged 12000xg for 15 minutes at 4°C. The aqueous phase was centrifuged and then transferred to a tube with 500 μ l of isopropyl alcohol for precipitation. After 15 minutes on ice, the RNA was pelleted by centrifugation at 40°C for 10 minutes. The RNA pellet was rinsed in 70 percent ethanol and centrifuged at 7500 xg for 5 minutes. The pellet was air-dried and 44 μ l of water were added.

Another method used was the Norgen plant RNA isolation (#26200, Norgen, Biotek) kit. Weighing around 50 mg of plant tissue and placing it in the mortar with liquid nitrogen and leaves were pestle. Then the sample was transferred to a 2 ml centrifuge tube and added 600 μ l lysis buffer C. Incubated at 55°C for 5 min. Mixing was transferred filter column and spin for 2 minutes at 20,000 x g (~14,000 RPM) after the centrifuge supernatant part was transferred to a new RNAase-free microcentrifuge tube. Added equal volume 96 % ethanol to the supernatant. Then 600 μ l sample was transferred to a spin column and centrifuged for 1 minute at 6000 rpm. The flowthrough was discarded. This part was repeated two times. After that washing step was applied, and 400 μ Wash Solution A was added column and centrifuged for 1 min at 14000 rpm. The flowthrough part was discarded and repeated, adding the wash solution part and centrifugation. This part was repeated three times. Lastly, flowthrough was discarded, and 50 μ l elution buffer was added to the column and centrifuge for 2 minutes at 2,000 rpm. The integrity of the RNA was checked on a 2% agarose gel. The NanoDrop spectrophotometer was used to quantify all RNA samples and check for contamination (BioSpec-nano #206-26300-48, JAPAN).

2.2.2. DNase I Treatment and cDNA Synthesis

RNase-free recombinant DNase I kit was used for DNase I method (# 4716728001 Roche, DNase I kit). RNA that calculated and 2 μ l was put into a tube. 2 μ l 10X incubation buffer and 0.25 μ l DNase I recombinant were added on RNA. Then water was added into the tube to make up to 20 μ l. After that was treated and incubated at 37 °C for 15 min. The reaction was stopped by adding 2 μ l of 0.2 M EDTA and heating to 75° C for 10 min.

A Thermo cDNA kit (# K2563, Thermo cDNA) was used for the first-strand cDNA synthesis. 2 μ l RNA, 1 μ l Random hexamer primer, and 9 μ l ddH₂O were added into the tube. After that, spin down was applied and incubated at 65 °C for 5 min. And at 70 °C for 5 min. Then it was incubated on the ice for 2 min. 4 μ l 5X buffer, 1 μ l RNase inhibitor, 2 μ l dNTP, and 1 μ l revert aid were added and incubated at 42 °C for 60 min. The cDNAs were stored at -20 °C.

2.2.3. Sample Preparation of RNA Sequencing

RNA samples were prepared for the Next Generation Sequencing (NGS). For NGS sequencing, two different RNA isolation techniques were used to evaluate which method would give better results. First, the samples taken from the leaf were isolated using the Norgen Plant Isolation kit (Cat. 31350), and secondly, isolation from the leaves was performed using Trizol. RNA samples obtained by both methods were used for NGS analysis. Approximately 35-40 μ l ~ 500ng/ μ l total RNA was used for measured NGS, which is enough for that. In this study, the lncRNA expression profile was analysed by the RNA-Seq method from two different tomato leaves (three biological duplicates were performed). Expression analysis of several of the identified lncRNAs was performed by quantitative reverse transcription-polymerase reaction (qRT-PCR). RNA samples, stored at -80 °C until needed, were placed in styrofoam boxes filled with ice blocks and sent to Macrogen (Seoul) by a courier company.

The bioinformatics analysis included three parts, quality check before preparing library, assignation of gene regions and differential gene expression analysis, and functional evaluation of the results.

2.2.3.1. Sample Preparation for RNA Quality

The library was created from the extraction obtained from RNA. After sending RNA samples to the Macrogen, RNA quality was measured. Quality control is RIN, and the RIN number should be >7. After the quality control (QC) was done, library construction was from the quality samples.

2.2.3.2. Library Construction

The cDNA sample's 5' and 3' adapter ligation created the sequencing library. In addition, the combination of "tagmentation" fragmentation and ligation dramatically increases the efficiency of the library preparation process. The parts attached with the adapter were amplified by PCR and purified with gel.

2.2.3.3. Sequencing

The library was loaded into a flow cell to create the cluster. The bridge amplification approach was used to amplify each fragment into separate clonal clusters. Templates were ready for sequencing after cluster formation was complete.

2.2.3.4. Generation Analysis of Raw Data

Raw imaging was generated using the primary analysis software Illumina sequencer, RTA (Real-Time Analysis). Base files were converted to FASTQ using Illumina bcl2fastq. Analysis of RNA-Seq data was performed by Sugenomix company.

2.2.3.5. Expression Validation of lncRNAs by Q-PCR Method

Takara SYBR Green (TB Green Premix Ex Taq II # RR82LR) kit was used for qPCR expression analysis. The expression level was checked in the Roche Lightcycler qPCR device, following the kit protocols. The qPCR method verified one up *003244833.1* and two down-regulated *183413.4* and *74190.3* lncRNAs selected among the lncRNAs determined due to Next Generation Sequencing analyses. The *Tubulin*, *18S*, and *Actin* genes were used as the housekeeping gene. Primers are given in **Table 2.4.** were designed utilizing the PrimerQuest tool of Integrated DNA Technologies. Analyses were performed using one-way Analysis of Variance (ANOVA). Tukey test was used to compare the means ($p < 0.05$). GraphPad Prism program was used for drawing graphics.

Table 2.1. Primers used in lncRNAs validation qPCR

Primer Name	Forward primer	Reverse primer
<i>Tubulin</i>	5'CCTGACAGCTTCTGC CATGT 3'	5'CATCTTCAGCCCAGT TGGTG 3'
<i>Actin</i>	5'AGGCAGGATTTGCT GGTGATGATGCT 3'	5'ATACGCATCCTTCTG TCCCATTCCTGA 3'
<i>18S</i>	5'GGGCATTTCGTATTTC ATAGTCAGA 3'	5'GTTCTTGATTAATGA AAACATCCT 3'
<i>74190.3</i>	5'CCTTTGCTCGTTTCG TCTCTA 3'	5'GCAAGGTGGAATGA CAACATC 3'
<i>183413.4</i>	5'TCACACGTCCTCTTC CTCTAT 3'	5'TCCTCCCATTGTAAC TCTTAGC 3'
<i>003244833.1</i>	5'CTTCGTTGAGAGGCC ATAGAAT 3'	5'AGCGGCATCAAGGT AGAAAG 3'

Table 2.2. The content of PCR mixture

Component	Volume	Final Concentration
I-taq™ Universal Sybr® Green Supermix	10 µl	1x
Forward Primer	1 µl	1 µM
Reverse Primer	1 µl	1 µM
Template DNA (5 ng)	2 µl	-
dH ₂ O	4 µl	-
TOTAL volume	12 µl	

Table 2.3. The experimental protocol for qPCR

Cycle	Temperature	Duration	Repeats
Pre-denaturation	95°C	30 seconds	1
Denaturation	95°C	5 seconds	40
Annealing	56°C	30 seconds	
Melt Curve	65-95°C	0.5°C increment at 2-5 seconds/step	
Hold	4°C	∞	1

3. RESULTS

This study consists of two parts, physiological and molecular experiments. In the first part of the thesis, grains of two different *Solanum lycopersicum* varieties in this study: Falcon and SC2121 seed, were sowed, and after 20 days, only H₂O (control) and cut the water (drought) for one week. After that, shoot and root height, chlorophyll amount, soil humidity, water content, soluble protein, and lipid peroxidation were determined.

The second part of this study contains next-generation sequencing analysis and determines gene expression of long non-coding RNAs levels on leaf samples. Total RNAs of drought stress applied leaves samples extracted with Trizol Reagent (Invitrogen) were sent to Macrogen company (in Korea). Next generation sequencing analysis was performed with RNAs. 1 up regulation and 2 down regulation gene controls were selected according to results from Macrogen was done by RT-PCR method. By using the $2(-\Delta\Delta Ct)$ techniques, relative quantification of lncRNA expression was assessed. The results were normalized using tomato Actin, Tubulin, and 18S rRNA.

3.1. PHYSIOLOGICAL EFFECTS OF DROUGHT STRESS ON TOMATO

3.1.1. Chlorophyll Amount

Table 3.1. Change to Chlorophyll concentration of leaves

Species	1. Leaf			2. Leaf			3. Leaf			4. Leaf			Average		
	1 st day	3 rd day	7 th day	1 st day	3 rd day	7 th day	1 st day	3 rd day	7 th day	1 st day	3 rd day	7 th day	1 st day	3 rd day	7 th day
Falcon Control	35,1	37,2	33,7	37,3	38,8	38,8	35,1	37,2	36,2	38,5	35,5	37,8	36,5	37,1	36,6
Falcon Stress	33,2	28,5	26,1	30,3	28,5	32,1	33,4	25,4	30,6	30,0	24,8	30,7	31,7	26,8	29,8
SC2121 Control	33,4	31,5	33,7	33,0	31,8	33,2	35,1	34,0	30,3	30,6	30,4	30,0	33,0	31,9	31,8
SC21121 Stress	30,6	27,1	24,0	30,8	28,1	27,1	28,5	24,9	27,2	29,3	28,2	27,0	29,8	27,0	26,3

The chlorophyll content of four selected leaves were measured at the end of the first, third, and seventh days after applying drought stress. After the measurements, it was observed that

there was a decrease and a slight increase in the amount of chlorophyll in the Falcon and SC2121 Control groups respectively. In Falcon Stress and SC2121 Stress groups, the amount of chlorophyll decreased on the third day, but there was no significant decrease in the measurements taken on the seventh day.

3.1.2. Measure of Soil Humidity

Table 3.2. Soil Humidity

Species	2 nd day	3 rd day	4 th day	5 th day	6 th day	7 th day
Falcon Control	Wet	Normal	Wet	Wet	Wet	Wet
Falcon Stress	Dry	Dry	Dry	Dry +	Dry +	Dry +
SC2121 Control	Wet	Wet	Wet	Normal	Wet	Wet
SC2121 Stress	Dry	Dry	Dry	Dry +	Dry +	Dry +

After the apply drought stress on the plant, the soil's moisture content was measured every day by soil moisture device. According to the measurement result, the soil's water value is normal and wet in the control groups. However, the Falcon and SC2121 stress group's grounds are dry, and this drought starts after the 2nd day. At the end of the seventh day, the dryness of the soil is at the highest level (Dry+).

3.1.3. Root and Shoot Height

Drought stress was studied in root and shoot heights on tomato plants. In terms of root and shoot, there was a significant difference between the control and treatment groups ($p < 0,01$). The chart below compares two different tomato varieties, shoot and root heights.

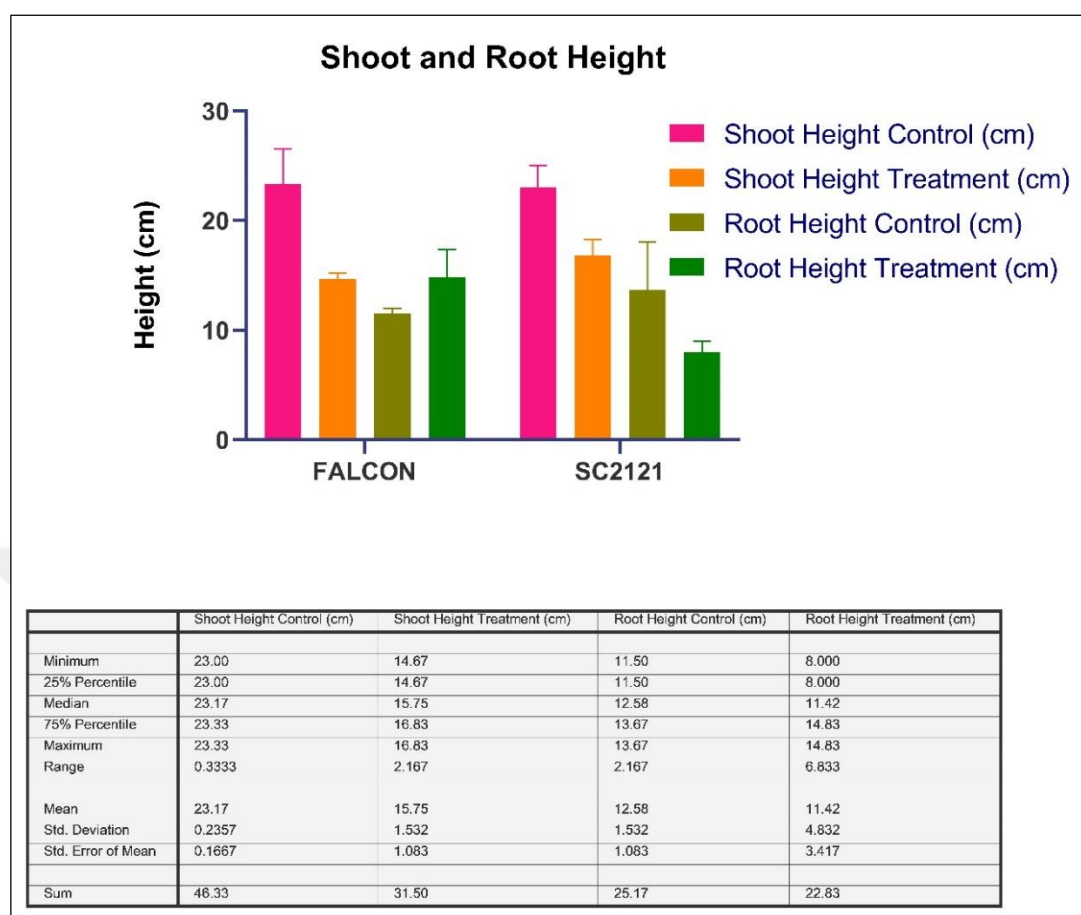


Figure 3.1. Effects of drought stress application on shoot and root

It was observed that the shoot and root length after drought stress the shoot length of falcon under drought stress was decreased by approximately 40% whereas SC2121 was 30% less. Conversely, while the root length of SC2121 under drought stress was reduced by approximately 40%, stressed Falcon root length increased by approximately 22% ($P < 0.05$).

3.1.4. Determination of Water Content (%)

At the end of the seventh day, the weights of the leaf samples taken from the plant and the control group from the drought-treated plant were weighed and dried in an oven at 65°C for two days. At the end of 2 days, the weights of the repetitions were measured, and the results were calculated according to the wet weights.

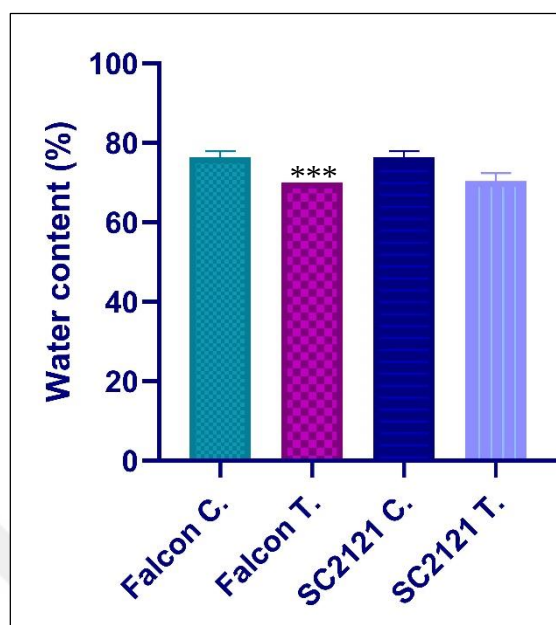


Figure 3.2. Effects of drought stress on leaves water content

Plant fresh and dry weights both decreased as a result of drought stress. According to water content results, Falcon's loss of water content is more than SC2121. Falcon leaves a loss of water content is more than SC2121, which was approximately 10%; SC2121 water content was reduced by approximately 4%. However, this decrease was a significant ($p=0.0008$).

3.1.5. Soluble Protein Content

The amount of soluble protein was measured from the leaves of Falcon and SC2121 tomato varieties, which were subjected to drought stress at the end of 7 days. The protein content of the control group and stress-treated leaf samples was measured by applying a BCA protein kit (Pierce)

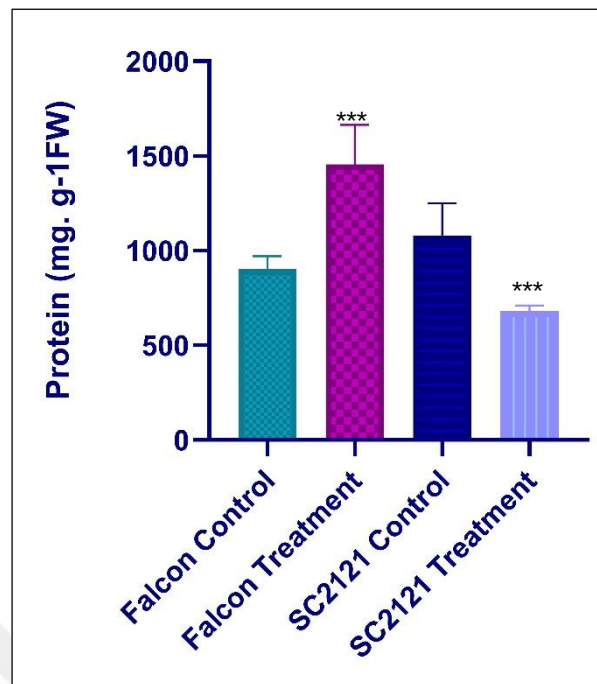


Figure 3.3. Effects of drought stress on soluble protein contents

When the soluble protein ratio of tomato plants exposed to drought stress for seven days was examined, interestingly, it was observed that the amount of soluble protein in the Falcon tomato genotype increased, and this was increased by approximately 40%. On the contrary, in the SC2121 tomato genotype, when drought stress is applied, it is seen that the number of soluble proteins decreases by approximately 40%; these changes are statistically significant ($P < 0.05$).

3.1.6. Lipid Peroxidation

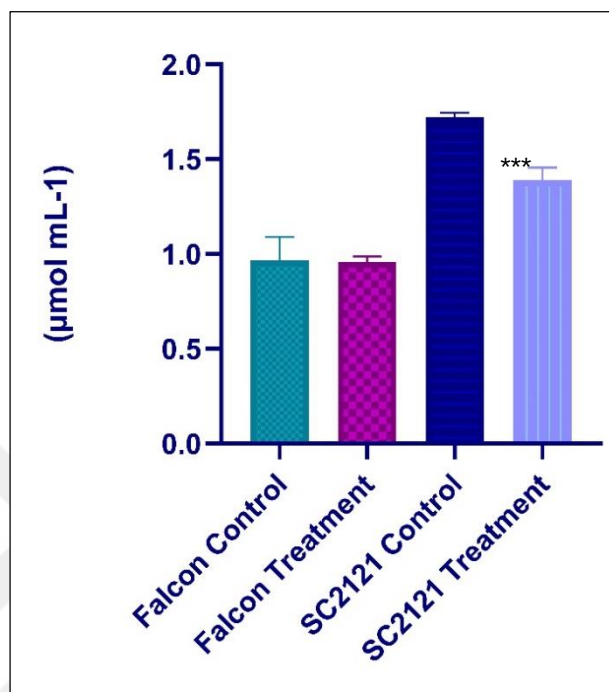


Figure 3.4. Drought stress's effects on leaf MDA

One of the by-products of polyunsaturated fatty acid peroxidation in cells is malondialdehyde (MDA). The overproduction of MDA is a result of a rise in free radicals that is associated with oxidative stress. Malondialdehyde (MDA) was calculated from the samples taken from the leaves of the tomato plant under drought stress and control conditions. Absorption values were measured at 600, 532, and 450 nm in a spectrophotometer, and calculations were made according to the lipid peroxidation formula. In the Falcon cultivar, the lipid peroxidation decreased slightly under drought stress but was not statistical decrease. The lipid peroxidation in the SC2121 variety was decreased more than in the Falcon variety by approximately 20%, and it was a statistically significant ($p < 0.05$)

3.2. MOLECULAR EFFECTS OF DROUGHT STRESS ON TOMATO

3.2.1. NGS (Next Generation Sequencing)

3.2.1.1. Library QS Result of RNA

Before starting the NGS analysis, the RIN number was analysed. 24 RNA samples sent to MacroGen were analysed using the RNA quality RNA RIN number (integrity number) Bioanalyzer 2100 device. To start the NGS analysis, the RIN value should be greater than 7.0. The values of our sent samples vary between approximately 2.6-8.1 RIN. Therefore, the NGS process could not be started due to the low RIN numbers value, and the samples were sent to the MacroGen company in dry ice using a different RNA isolation kit. Since the RIN values of the samples were sent for the second time and the samples' RIN numbers were between 6.5-8.0, NGS analysis was started. Checked the quality control of the final library. When we look at the quality control results, the first peak is lower (Figure 3.5), and the other peaks show 5S, 18S, and 28S in the electro fragments at the top. The RNA is of good quality if the 18S and 28S peaks are high. That means a high RNA integrity number (RIN). The RIN quality rating is ranked from 1 to 10. Generally, the samples with a RIN value of 7 and above for NGS continue to work. As it decreases from 10 to 1, it is seen that there is an increase in degradation.

Table 3.3. RNA samples for NGS analysis

Falcon Controls	Falcon Cont1	Falcon Cont2	Falcon Cont6
Falcon Stress	Falcon Stress3	Falcon Stress5	Falcon Stress6
SC2121 Controls	SC2121 Cont1	SC2121 Cont4	SC2121 Cont6
SC2121 Stress	SC2121 Stress4	SC2121Stress5	SC2121 Stress6

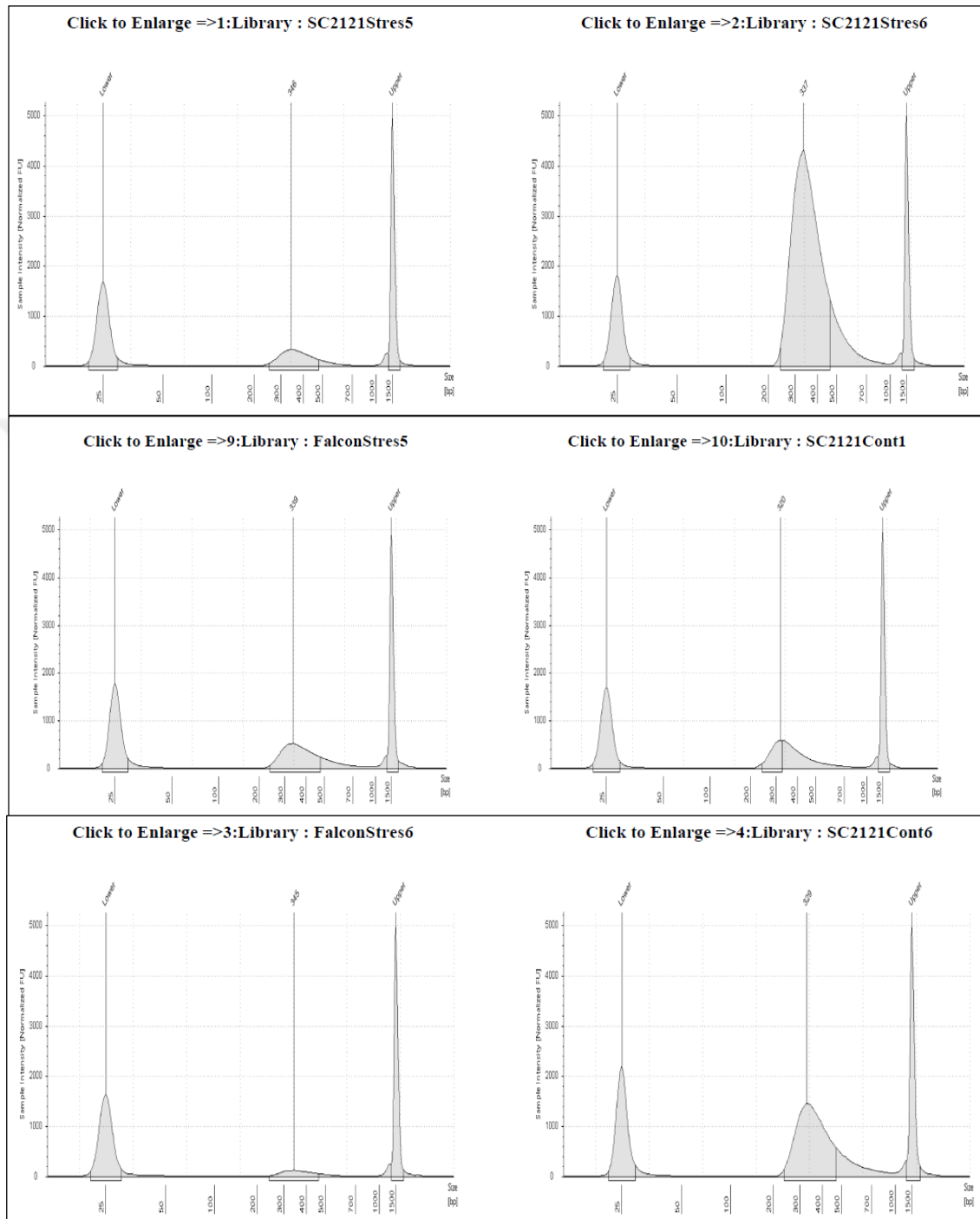


Figure 3.5. SC2121 Stress5, SC2121Stress6, Falcon Stress5, SC2121 Cont1, Falcon Stress6, SC2121 Cont6. RNAs Samples quality controls

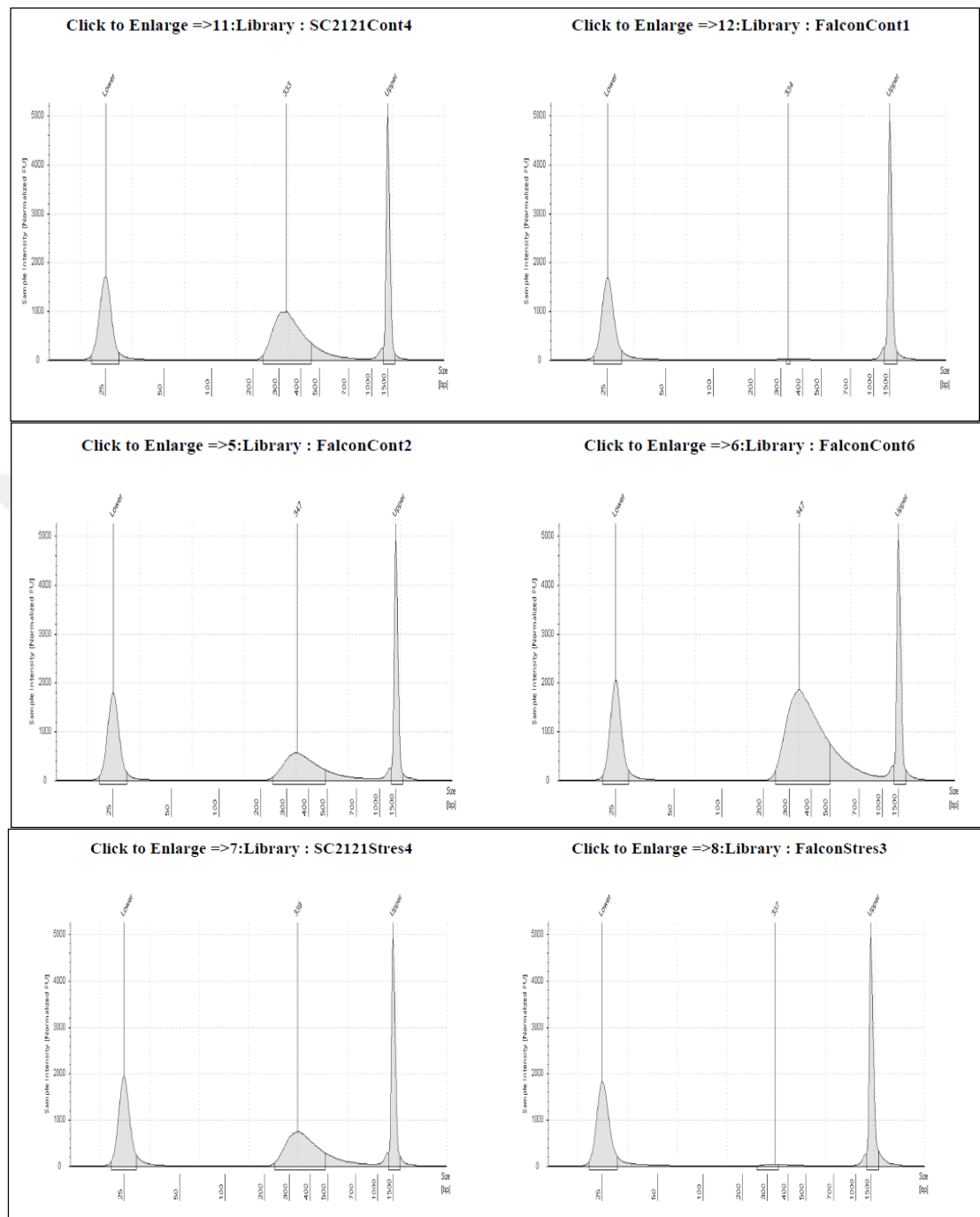


Figure 3.6. SC2121 Cont4, Falcon Cont1, Falcon Cont2, Falcon Cont6, SC2121 Stress4, Falcon Stress3 RNAs Samples quality controls

3.2.1.2. Raw Data Statistics

Total base count, read count, GC (%), Q20 (%), and Q30 (%) were calculated for 12 samples. For example, 64,954,522 reads were produced on FalconCont1, with total read bases of 6.6G bp. This GC content (%) is 42.46%, and Q30 is 95.22%.

Table 3.4. Raw data stats

Sample ID	Total read bases (bp)	Total reads	GC(%)	AT(%)	Q20(%)	Q30(%)
FalconCont1	6,560,406,722	64,954,522	42.46	57.54	98.27	95.22
FalconCont2	7,644,797,262	75,691,062	42.53	57.47	98.48	95.65
FalconCont6	6,515,184,174	64,506,774	42.75	57.25	98.48	95.66
FalconStres3	6,069,034,450	60,089,450	43.16	56.84	98.28	95.22
FalconStres5	7,089,862,660	70,196,660	42.6	57.4	98.27	95.52
FalconStres6	6,563,033,126	64,980,526	42.44	57.56	98.21	94.87
SC2121Cont1	8,217,794,704	81,364,304	42.99	57.01	98.71	96.04
SC2121Cont4	6,675,559,448	66,094,648	42.25	57.75	98.77	96.0
SC2121Cont6	6,437,947,050	63,742,050	42.56	57.44	98.59	95.8
SC2121Stres4	6,940,206,112	68,714,912	42.62	57.38	98.48	95.69
SC2121Stres5	6,286,239,596	62,239,996	42.75	57.25	98.72	96.07
SC2121Stres6	7,043,687,076	69,739,476	42.66	57.34	98.81	96.03

Raw data shows name of the samples, total number of bases sequenced, GC (%) and AT (%) shows content of GC, Q20(%) and Q30(%) Indicates the proportion of bases with a quality greater than 20 and quality greater than 30.

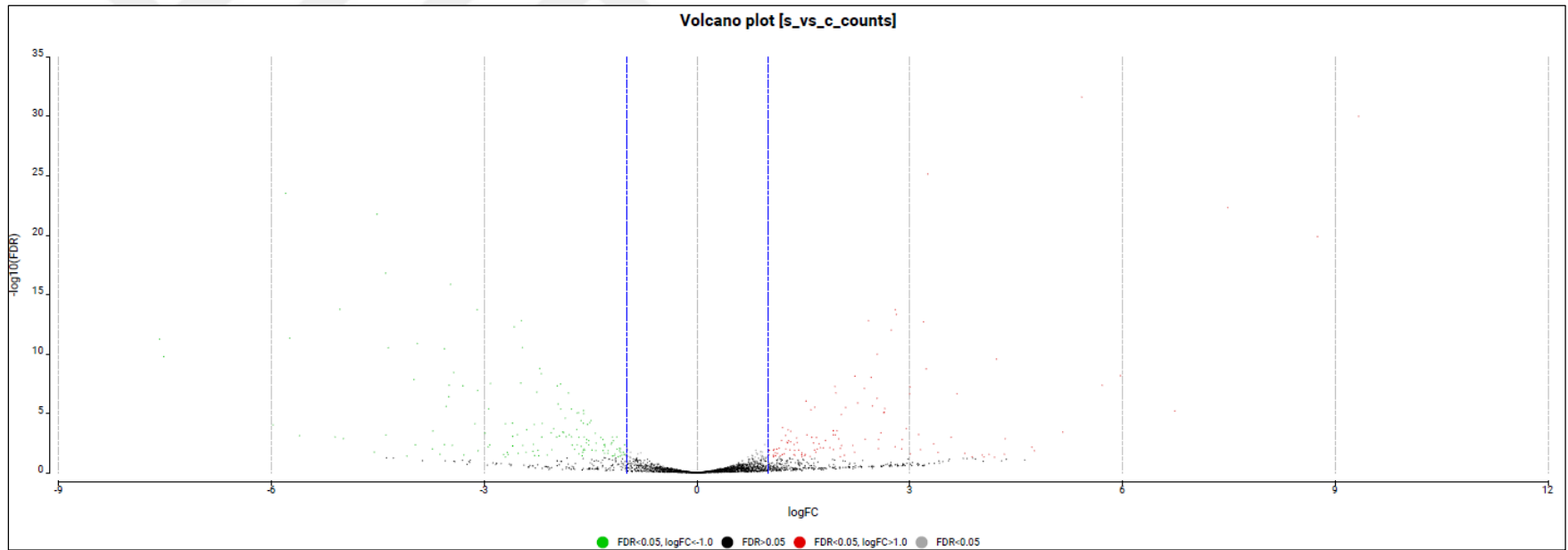


Figure 3.7. Analysis of lncRNAs in tomato leaves using a differential expression volcano

Volcano plots (figure 3.6) are significantly used to display the results of RNA sequencing. A volcano plot is a graph that shows the statistical change of change and the magnitude of the change. It provides rapid relative identification of genes that increase statistically significantly. In a volcano plot, the genes that show the most increases are shown on the right side of the graph (red color), and the genes that decrease the most are shown on the left side of the chart (green color). In addition, statistically significant genes are found towards the top.

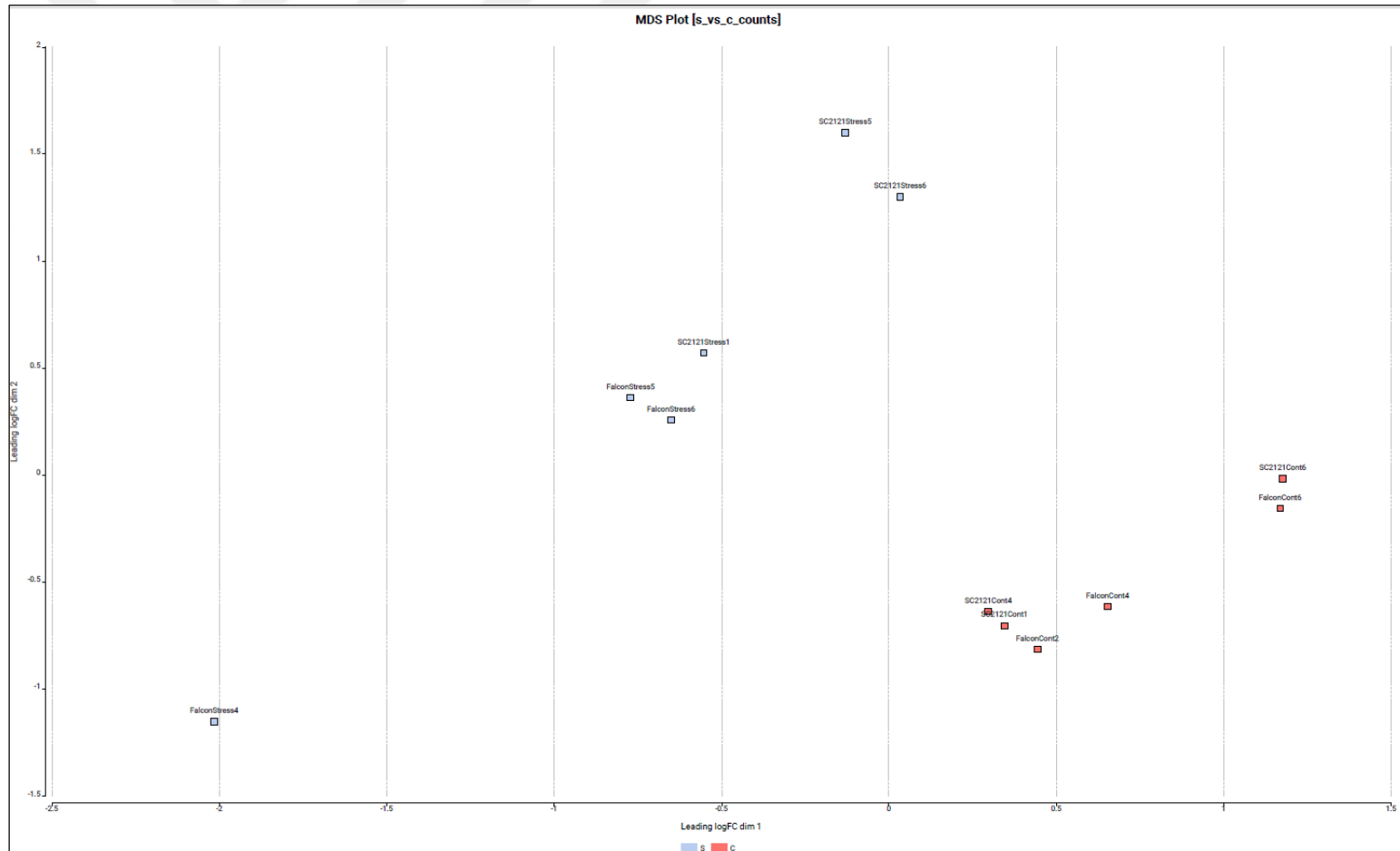


Figure 3.8. MDS (Multidimensional Scaling) plot displays the similarity between control and treatment samples.

It is seen that those indicated in red are the control group, those shown in blue are the stress groups, all control groups are close to each other in the same region, and the stress groups are in a different region from the different control group.

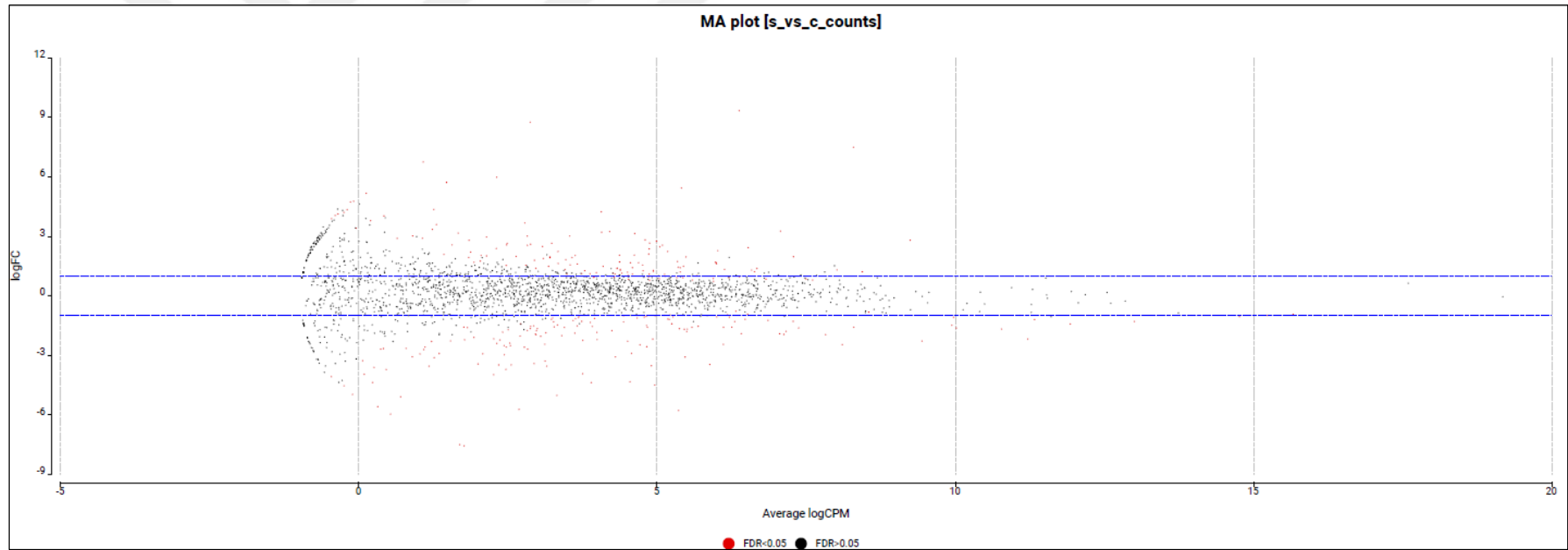


Figure 3.9. MA plots demonstrate the mean expression versus log fold change between the control and stress groups.

The MA graph (figure 3.8) shows the variance between the log ratios of the two variables. The value "M" means minus on the log scale. MA plot diagram of the differential expression analysis of SC2121 and Falcon stress. Blue line < 0,05 fold change. Mean average FPKM values. Significantly up regulated genes (red), down regulated genes (green, black).

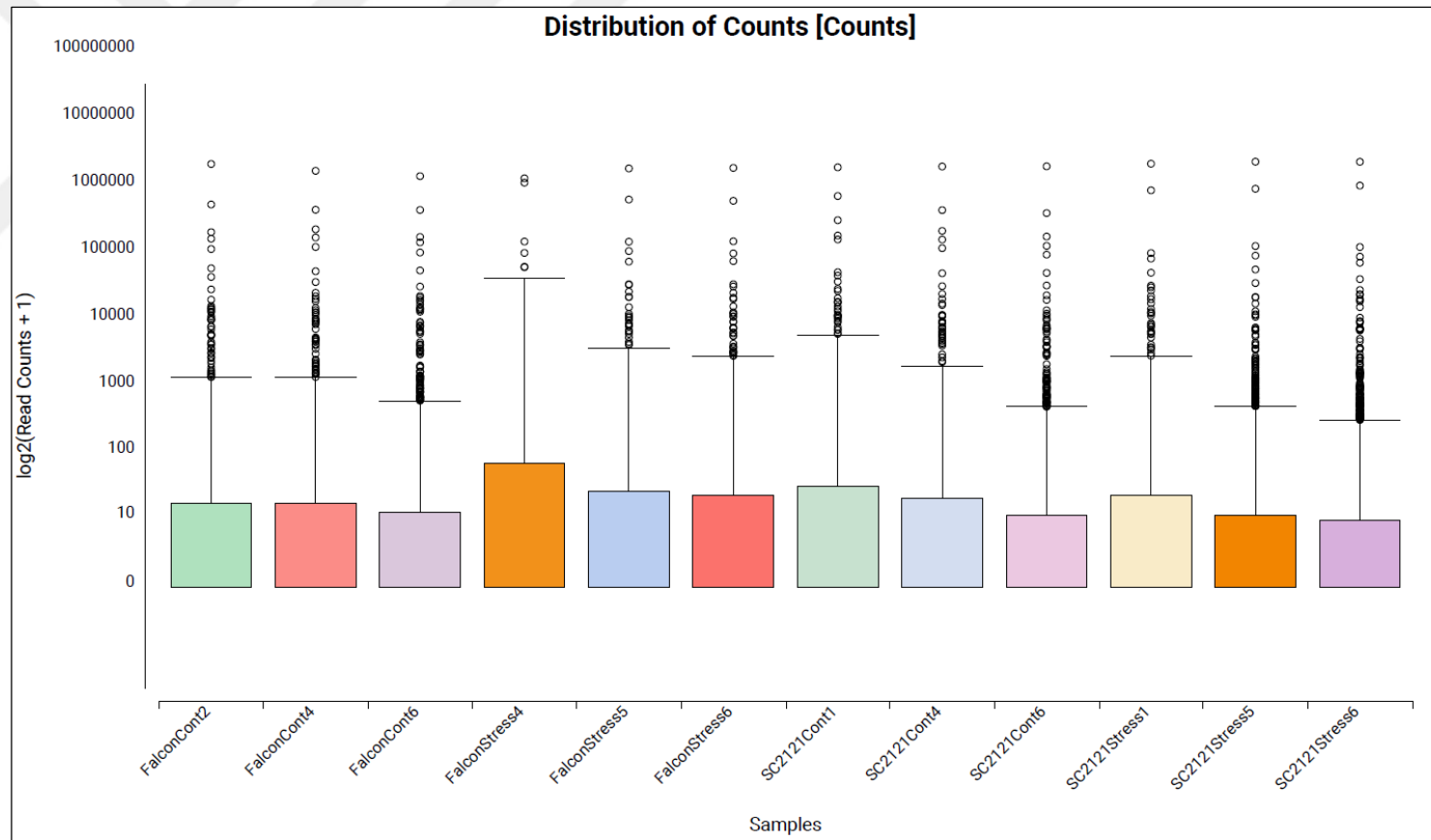


Figure 3.10. The box plot showing the expression level of lncRNA. RNA sequencing expression distribution shows a boxplot. The raw distribution is log2 cycle applied.

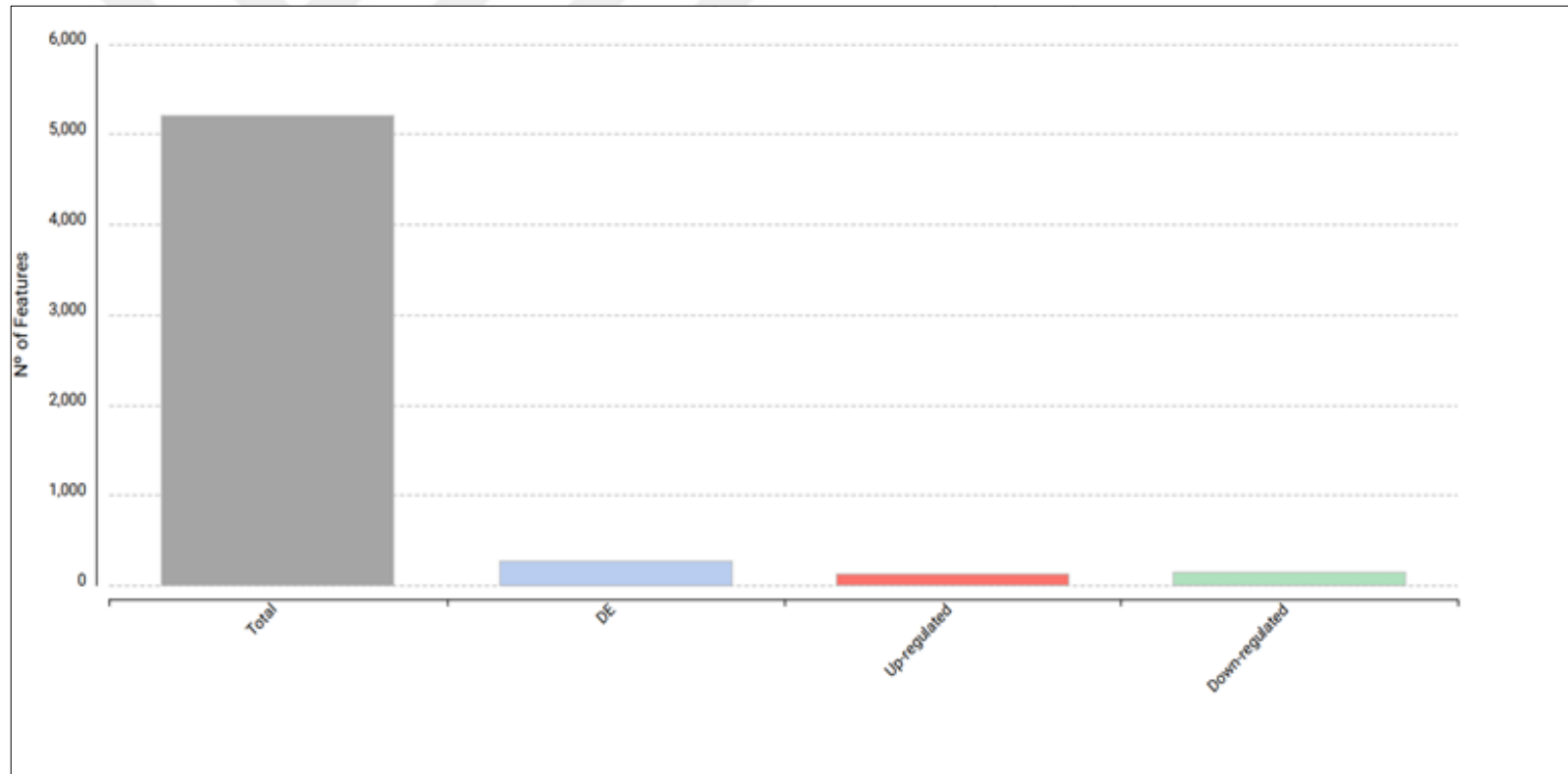


Figure 3.11. Histogram showing differential expression lncRNAs upregulation and downregulation of SC2121 and Falcon tomato varieties

Differential gene expression is a widespread implementation of RNA-sequencing data. Number of total features 5,208; filtered features 2,841; after filtering 2,367 number of analysed. 124 Up-regulate genes are showing red, and 145 down-regulated genes are showing.

Table 3.5. Differentially expressed upregulate and downregulate lncRNAs

Tags	lncRNAs Name	logFC	FDR
UP	rna-XR_183086.4	1.01091984056702	0.0131168028059807
UP	rna-XR_003243960.1	1.01680986109609	0.0314068790611361
UP	rna-XR_743618.3	1.02034403732068	0.0129526664711311
UP	rna-XR_743350.3	1.07829431121101	0.0327580751666798
UP	rna-XR_003245449.1	1.08068138723109	0.0458575502876423
UP	rna-XR_002026453.2	1.08216555564615	0.0131654878947899
UP	rna-XR_003244833.1	1.08535821158563	0.0396878101157929
UP	rna-XR_183182.4	1.09108423833026	0.0107204372401571
UP	rna-XR_003246012.1	1.10645854276794	0.0212666897384986
UP	rna-XR_003247036.1	1.11089329988334	0.0362708441830248
UP	rna-XR_002028295.2	1.13082968188781	0.0102647971929561
UP	rna-XR_182937.4	1.13558361581727	0.0231360185474599
UP	rna-XR_183089.4	1.16202186295706	0.008629391353323
UP	rna-XR_742751.3	1.17979340491923	0.0370156875352465
UP	rna-XR_183317.4	1.19656193028854	0.00191209271039897
UP	rna-XR_743635.3	1.20759221423883	0.0294959138716712
UP	rna-XR_741135.3	1.21163840483715	1.57862138172567E-4
UP	rna-XR_003247047.1	1.23205071668361	0.0259441575431359
UP	rna-XR_740042.3	1.25390807288614	7.29655673949051E-4
UP	rna-XR_739428.3	1.25692297305987	0.00993244578345582
UP	rna-XR_003246067.1	1.2739694334799	0.00271560391497826
UP	rna-XR_741901.3	1.28070897273036	0.00303866975719942
UP	rna-XR_741246.1	1.28834595066546	2.44108832392249E-4
UP	rna-XR_002026068.2	1.29447201870645	0.00203517747983748
UP	rna-XR_003248241.1	1.31446231608331	0.0121816531984388
UP	rna-XR_182724.4	1.32300781455441	0.00249314872265332
UP	rna-XR_739012.3	1.32556479810999	3.25998060514223E-4
UP	rna-XR_002027552.2	1.36952438065126	0.0296513137305492

UP	rna-NR_144505.1	1.37205053882837	0.00416415626092645
UP	rna-XR_003247517.1	1.38104149018645	0.0131654878947899
UP	rna-XR_742482.3	1.38370612191876	0.034680696606595
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UP	rna-XR_003246188.1	1.52160140547247	0.0365557032964785
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UP	rna-XR_741037.3	1.5535396517823	6.48433184953035E-4
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UP	rna-XR_740893.3	1.78444645636904	0.00810006266058836
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UP	rna-XR_182989.4	1.94941627219185	5.50223872016872E-8
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DOWN	rna-XR_003246821.1	-1.03648971672156	0.0187228838477479
DOWN	rna-XR_740222.2	-1.02416171501391	0.0311136229643756
DOWN	rna-XR_003248212.1	-1.01675402619689	0.00443392692043052
DOWN	rna-XR_003244982.1	-1.01566142303129	0.0283398519391916

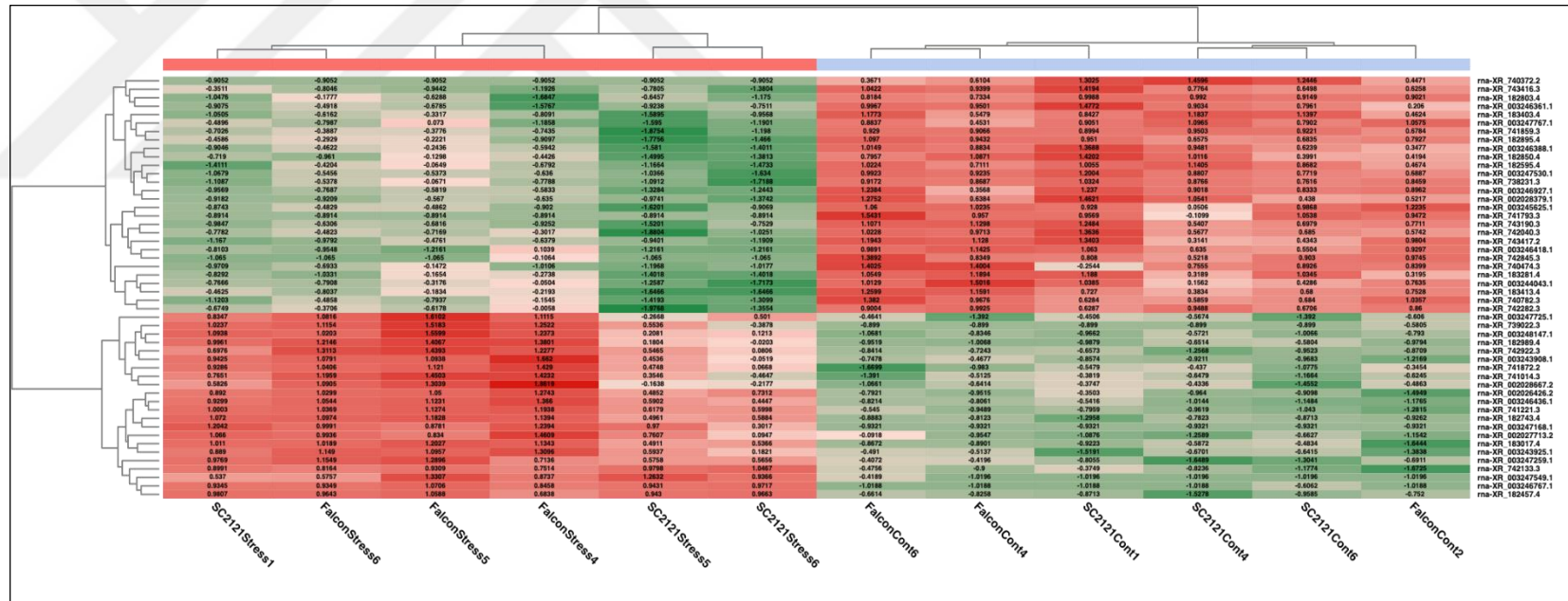


Figure 3.12. Heat Map presentation of relative expression levels of differentially expressed lncRNAs between different tomato varieties

This heat map shows a gene expression between different tomato varieties. Each column includes changing gene expression for only one sample. The high expression level is red colour, and the low expression level is green colour. For example, XR_003247168.1, XR_003247549.1, and XR_002027713.2 expression levels are very high on falcon stress; XR_183413.4 expression level is low in SC2121 stress group.

3.3. EXPRESSION VALIDATION OF LNCRNAs BY Q-PCR RESULT

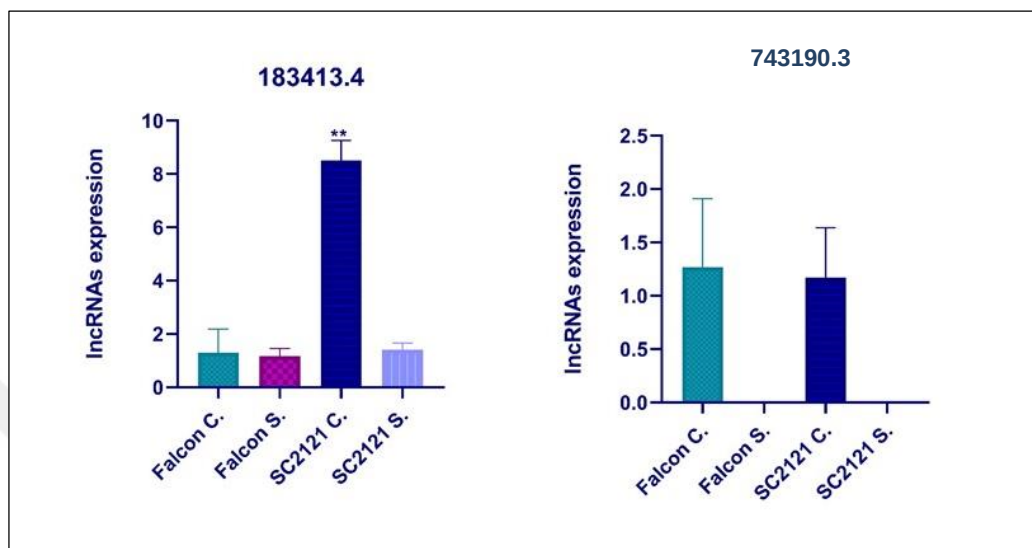


Figure 3.13. Expression analysis of XR_183413.4 and XR_743190.3 down regulate DELs by RT-qPCR

The median of three biological replicates and three technical replicates, together with their respective standard deviations, are shown in bars. The symbols * or ** indicate statistical differences for RT-qPCR data, ($P < 0.05$).

According to the NGS result, XR_183413.4 and XR_743190.3 expressions are down-regulate, so when we validate these genes on RT-qPCR, our results are coherent with NGS. In the NGS expression results, while expression is visibly low in the SC2121 stress cultivar in XR_183413.4, the Falcon stress type decreases less. XR_743190.3 is also visibly down-regulated under stress in both Falcon and SC2121 varieties.

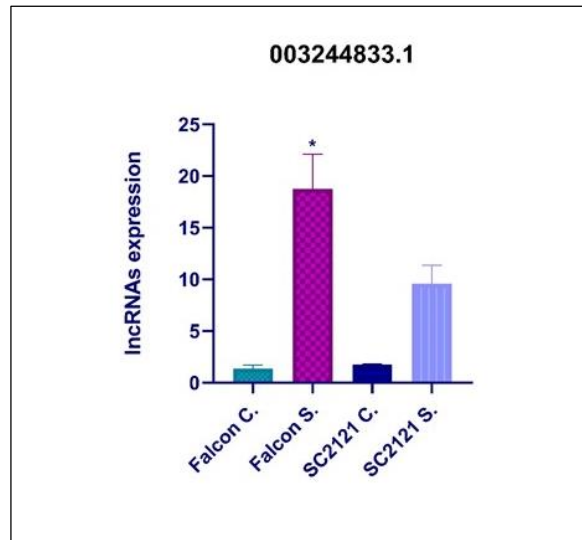


Figure 3.14. Expression analysis of XR_003244833.1 up regulate DELs by RT-qPCR.

Bars show the median of each set of 3 technical and biological duplicates, together with their respective standard deviations. Results from RT-qPCR are indicated by * or ** if there are significant differences and ($p < 0.05$). XR_003144833.1 lncRNA expression up-regulates both Falcon and SC2121 cultivars, for the Falcon stress expression is higher than the SC2121 stress type.

4. DISCUSSION

All living things on earth are seriously affected by climate changes. Climate change is a situation that changes the typical climatic conditions that occur artificially or naturally. Climate change is emphasized among the UN sustainable development goals, and it is argued that action should be taken to minimize the effects of global change. The resulting climate change seriously affects the yield and diversity of plants. The most significant change due to climate change is the drought that occurs due to the decrease in precipitation [11]. One of the most significant elements influencing agricultural productivity is drought. Water deficiency restricts the plant's growth by inhibiting the plant's respiration, photosynthesis, and stomatal movement. In response to these restrictions, plants activate many mechanisms to reduce the impact of stress, including morphological and structural changes, activation of genes relevant to drought, and hormone production [19]. Drought stress causes various biochemical and physiological changes during plant development. In this thesis, we checked molecular and physiological experiments eventuate under drought stress on tomato plants. Firstly, two different tomato varieties were chosen to determine chlorophyll amount, root and shoot growth, water content, soluble protein content, lipid peroxidation under control, and drought stress circumstance.

The amount of chlorophyll is one of the photosynthetic properties strongly affected by water deficiency. In case of water deficiency, the opening and closing of the plant's stomata directly affect the amount of chlorophyll [22]. The decrease in chlorophyll causes a decrease in plant productivity [121]. Chlorophyll amount was measured during the seven-day drought application. For this analysis, mature leaves were used. First-day chlorophyll amount was measured and respectively Falcon and SC2121 varieties. When 3rd-day and 7th-day chlorophyll was measured, chlorophyll was observed that the amount of chlorophyll decreased, and the decrease in the amount of chlorophyll was specifically in the SC2121 Stress group. SC2121 Stress first leaf amount chlorophyll was 24.0 and second leaf is 27.1. Young leaves had more chlorophyll owing to enzyme activation in moderate responses, but older leaves under drought had less enzyme inactivation. The reason for this is the decrease in carbon dioxide intake due to the decrease in photosynthesis in the stomata [122].

The roots direct interact with the soil and get the water and minerals from the ground. In addition, these are the most sensitive and significant organs to drought stress. For this reason,

the roots are vital organs for understanding water deficiency effects. The height of plants exposed to water scarcity is associated with cell growth and is severely affected by drought. Stem growth and leaf growth are more susceptible than root growth in plants under drought. In the first times of drought, plants slow down stem elongation and trigger root development to reach water more efficiently. Drought stress affects cell expansion due to decreased turgor pressure. Turgor pressure is one of the factors affected by drought stress. Cell growth in plants occurs when the turgor pressure exceeds the cell wall yield threshold [123]. In a study conducted on melons in 2015, it was determined that drought significantly affected the diameter of the main stem. If the drought lasts for a long time, both root development and stem development stop [44,124]. Rice changed its roots under drought stress and provided longer and deeper growth in the soil, activating the drought stress avoidance mechanism. Contrary to this, some studies have shown that root length decreases under drought stress [125]. In this thesis we analysed tomato root and shoot height under drought stress. After the drought stress, Falcon Tomato shoot height decreased significantly while root length increased. Falcon tomato shoot height decreased significantly according to the control group, which decreased by approximately 8 cm. In comparison, root length increased by 4 cm. These results can indicate a drought avoidance mechanism. When we look at the SC2121 tomato variety, shoot height and root length decreased significantly. This result demonstrated that SC2121 variety are negatively affected by drought stress.

Drought stress has a significant impact on the plant-water relationship. Water content (WC) (leaf water potential) is among the features affected by drought. When we look at the plant that is exposed to water stress in general, it is seen that the amount of water in the leaves and roots decreases [126]. While the water content in the leaves of sufficiently irrigated plants is around 90%, the water content of the leaves in the medium stress condition is between 40-60%, and the water content in the leaves of the plants exposed to intense stress is below 40% [127]. For the water content study, different results have been observed in tomato two cultivars used in this study. Water content of Falcon and SC2121 demonstrated reduction. The reduction in water content varies with both crop type and stress level. This situation is also related to the drought tolerance of the plant. For instance, when faced with drought stress in drought-tolerant genotypes, it was observed that their water content was preserved, and their leaves faded late [128]. In a study conducted by Kuşvuran on melons in 2010, drought stress was examined in 31 different melon genotypes, and it was observed that water

content decreased in many genotypes [42]. In the study on bean genotypes, drought stress was applied at different levels, and the water content decreased significantly according to the drought level [129]. Aranjuelo et al. In their study on alfalfa (*Medicago sativa L.*) in 2010, after applying drought stress for seven days, the water content was examined, and it was observed that there was a decrease in water content from 76% to 51% [39]. When we compare all these studies with our water content result, we see that there is not much water loss. The reason for this may be that the tomato type used is different, the plants are not exposed to enough stress or the drying process applied to the leaves for drying weighing is not sufficient.

Other functional groups that play a role against drought stress of plants are proteins. In many plants, various responses occur in cells in water deficiency, and differences in protein expression, synthesis, and accumulation, generally mediated by the hormone Absciscic acid, have been observed. Drought stress can also cause qualitative and quantitative changes in different plant species [130]. LEA proteins, osmotins, and heat shock proteins are produced to protect cells against drought [131]. Plants grown under the influence of various environmental factors cause changes in the amount of protein with a decrease in protein content, which results in a decrease in protein content due to the increase in Pro content. These changes can be attributed to protein hydrolysis causing Pro aggregation. In previous studies, the accumulation of dehydrin-like proteins in wheat and soybean is detected in the leaves and roots of plants exposed to drought stress. This shows that the plants are protected from further dehydration damage [130]. In this thesis, soluble protein analysis has been applied to two different tomato varieties. Falcon tomato variety total soluble protein increased significantly while SC2121 variety total soluble protein declined. This result may indicate that the Falcon tomato variety is actively responding to drought stress, and that SC2121 variety is susceptible. In the drought study on maize genotypes in 2007, the amount of soluble protein first increased with the decrease in water potential. Then the amount of soluble protein decreased [132]. In the study conducted by Kosar et al. in 2020, total soluble protein has been decreased in sunflowers under drought stress [133]. It has been understood that the soluble protein concentration depends on the nature of the plant species and the type of tissue under stress. When studies on drought are examined, there are references to the build-up of dehydrin proteins in several plant species, including grapevine, wheat, and

soybean. Plants under stress might accumulate dehydrin-like proteins in their roots or leaves [32].

Plant metabolism includes lipid anabolism and catabolism, and lipid metabolism is also affected by water deficiency. Lipids are one of the essential parts of the cell membrane and prevent the division of cells under drought stress. In a study in *Arabidopsis*, essential lipids such as monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) were elevated in the deficiency of water [134]. Lipid peroxidation causes cell death due to destroying the cell membrane's aggregation. The superoxide radicals (O_2^-) and hydrogen peroxide (H_2O_2) cause increased lipid peroxidation. Oxidants initiate lipid peroxidation by reacting with polyunsaturated acids. Lipids are one of the essential parts of the cell membrane and prevent the division of cells under drought stress. The end product of lipid peroxidation is malondialdehyde (MDA) and ethane. The resulting MDA affects the ion exchange in the membranes and causes cross-linking of the compounds in the membrane. It causes undesirable results, such as changes in enzyme activity and ion permeability [42,133]. In Xu's study in 1995, wheat was exposed to drought stress to observe lipid peroxidation. In the study, it was observed that MDA content increased significantly after drought [123]. In a study in *Arabidopsis*, essential lipids such as monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) were elevated in the deficiency of water. Since drought stress deactivates the enzymes responsible for the metabolism of these lipids, their amounts have increased. In this study, lipid peroxidation has not been changed with drought stress in the falcon variety but lipid peroxidation has been decreased with drought stress in the SC2121 genotype. Shafiq et al. showed that with the increase of abiotic stress, oxidative free radicals that degrade fatty acids are produced, and lipid peroxidation increases. A study on sunflower stated that MDA concentration was high, similar to the studies on wheat [135,136]. In many studies conducted under stress, it was observed that the MDA level increased under drought stress. According to the results in the literature, under drought stress, the membrane structure deteriorates and MDA is released and MDA increases. However, according to our results, MDA is decreasing. This may be because the plants are not sufficiently drought stressed and they are not so dehydrated that their membrane structure can damage.

lncRNAs have been previously identified in many plant groups such as *Arabidopsis thaliana*, maize, barley, rice, tea and wheat [63,137,138]. It has been determined that lncRNAs have

functions in tissue development under many stress groups of plants such as heat, cold, and drought. Thanks to these discoveries, it has been stated that lncRNAs have made significant contributions to agricultural studies. For example, a study in rice revealed that the intergenic lncRNA PMS1T (Photoperiod-sensitive genic male sterility) and LDMAR (Long-day specific male-fertility associated RNA) were male sterile while applying the antisense transcript LAIR to rice was controlled and controlled for state control. The most important finding is that they try to adapt to changing environmental conditions [139,140]. In a study conducted in cassava under drought stress, 51 lncRNAs associated with drought were detected and confirmed by qRT-PCR. Confirmed lncRNAs were determined to regulate ABA and ethylene signalling pathways[141]. In a similar study, lncRNAs were investigated under drought stress in switchgrass. It was observed that XLOC_053020, XLOC_014465, and XLOC_033252 were involved in ABA synthesis, and XLOC_074836 was involved in ethylene signalling and up-regulated [142].

In studies on non-coding RNAs, many features of lncRNAs have been identified, and it has been stated that lncRNAs are associated with drought stress. A study by Liu et al. on *Arabidopsis* found that drought stress and abscisic acid (ABA) were treated together, and At5NCO5682 expression changed drastically. In this case, it was stated that lncRNAs might play a role in the plant's resistance to drought stress [143]. In a study by Quin et al., drought-related lncRNA (DRIR) was discovered after drought stress and ABA treatment. They showed that the DRIR lncRNA would regulate ABA signalling and water transport stress-related genes, making plants amenable to drought and salt stress [144]. Lu et al. applied drought stress to cotton and studied the functions of lncRNAs with RNA-seq technology. It was found that the lncRNAs, XLOC_063105, and XLOC_115463 may play a role in regulating the drought response pathways of plant hormones [145].

Recently, the roles of long non-coding RNAs in plants are becoming increasingly important. The limited number of studies on ncRNAs have shown that ncRNAs have crucial roles in the developmental processes of plants [116]. The role of lncRNAs under drought stress has not been adequately studied. Nowadays, RNA-seq technologies allow us to follow the evolution of various ncRNAs in response to stresses. Thus, it allows us to understand the functional mechanisms of RNAs better. In a study conducted in 2022, 67,770 lncRNAs were identified in tomato plants, and 4716 lncRNAs were annotated by CANTATAdb [46].

This thesis study, 5,208 lncRNAs were obtained from each sample 60 million NGS readings for Falcon and SC2121 each tomato varieties. The leaf samples for NGS analysis were sent to the Macrogen company in Korea. However, in the analysis, since the library process was passed without applying the DNase I process, this situation seriously affected our results as too much nuclear particularly chloroplast DNA were taken into the library. Due to this situation, no more lncRNAs and novel lncRNAs could be obtained. It was impossible to repeat the experiment because the budget was insufficient, and the company where the sequencing was made was in Korea.

From these data, 269 lncRNA were differentially expressed, (Table 3.4) 124 lncRNA up-regulated, and 145 lncRNA down-regulated. These 269 lncRNAs can be grouped according to their genome location into antisense lncRNAs, sense lncRNAs and intergenic lncRNAs. The top70 grouping and functions of lncRNAs with the highest expression were examined. 45 lncRNAs intergenic, 19 lncRNAs sense and 6 lncRNAs antisense.

lncRNAs expressed according to differentially expressed profiling analysis are given in (Figure 3.11) The results showed that the expression level and Falcon stress expression level are higher than in SC2121 tomato cultivars subjected to drought stress.

To understand the relationship between the expression of lncRNAs sensitive to drought stress and their potential target genes, 12 lncRNAs whose functions and biological processes were determined in the literature were selected. Selected genes' biological process, molecular function, and cellular component analyses were performed. Under water deficit, XR_003247767 sense lncRNA is upregulated, and Uridinetriphosphate is required in sucrose synthesis. (UTP), Cytidine required in the synthesis of phospholipids. It takes part in the synthesis of triphosphate (CTP). Cytidine triphosphate (CTP) is required for DNA, RNA, and phospholipid biosynthesis [146]. XR_003247767 lncRNA plays a crucial role in purine and pyrimidine nucleotide biosynthesis (adenylate kinase). Upregulation of this gene has shown that it can adapt to water-deficient conditions by increasing ATP energy supply and reducing oxidative damage. Reducing oxidative damage by supplying ATP energy through promoting purine and pyrimidine nucleotide biosynthesis and scavenging of superoxide radicals may be closely associated with increased drought tolerance [147,148].

Another sense lncRNA is XR_182895 and upregulated, the molecular functions are RNA binding and protein dimerization activity. RNA binding proteins (RBPs) are the most

important regulatory mechanism of lncRNAs that mediate post-transcriptional gene regulation [149]. Lorkovic's study in 2019 exhibited that some RBPs have regulatory roles against stress responses. In particular, he stated that RBPs are among the proteins that regulate the ABA signalling pathway [150]. XR_182895 gene also plays a role in ribonuclease T2 activity. Ribonucleases (RNases) are non-base acidic endoribonucleases linked to the RNase T2 family [151]. A study on rice investigated the RNase T2 family and found that RNase Os encodes an active enzyme purified from rice bran. The expression of GAR-RNase protein, known as the barley enzyme, is induced by gibberellins and suppressed by abscisic acid. Another rice gene belonging to the RNase T2 family was identified from rice leaves exposed to water deficiency. The protein, designated RNase DIS, was highly induced and appeared to have the same homology as RNase T2 [152,153]. In another study, drought-associated S-like ribonuclease was up-regulated in rice under drought stress [154].

XR_003246388.1 sense lncRNA is upregulated gene in SC2121 stress expression is high than Falcon stress. Molecular function of this lncRNA protein dimerization activity. Recent studies show that protein dimerization can have a very important role in the regulation of factors such as ion channels, enzymes and transcription. K channel activity mediated by ABA-induced Ca-dependent and independent pathways is required for stomatal opening and closing. XR_182895. may play a role in the opening and closing of stoma by controlling K⁺ levels [117]. The other function of this gene is, it is a molecular function in which any gene product binds non-selectively with DNA (DNA binding).

XR_002028379.1 is a sense lncRNA and upregulate gene, biological process is cetyl-CoA metabolic process. Acetyl-CoA is the precursor of phytochemicals that are important for plant growth and development. A study in Arabidopsis has proved that acetyl-CoA carboxylase (AAC1) is necessary for the development of the embryo [155]. In a study on the tea plant, the accumulation of ACC1 was down-regulated in response to drought stress, indicating that it could increase resistance to water deficiency by inhibiting the accumulation of ACC1 [156]. Acetyl-CoA acts as a signal regulatory response to various abiotic stresses and growth. Histone acetylation plays a role in the epigenetic modifications that cause this reaction [157]. Molecular function of gene acyltransferase activity, acyltransferase is one of the crucial enzymes that maintains the structure of membrane lipids and enables the conversion of phytosterols to sterile esters [158].

XR_003247725.1 antisense and XR_003243925.1 sense upregulate lncRNA molecular functions are ubiquitin-protein transferase activity. Ubiquitin is highly conserved in humans, yeast, and plants and is the same in all. Ubiquitin has a significant role in the continuation of cellular functions, and these functions have a wide range of functions such as cell signalling, protein processing, and DNA repair. Ubiquitins are compounds that respond to developmental and environmental responses in plants. In particular, their responses to biotic and abiotic stress require ubiquitination and are modulated by ubiquitin. It is also widely used as a component that increases gene expression [159,160].

XR_741872.2 gene is associated with the ATP binding molecular function and it is antisense lncRNA. ATP binding cassette proteins (ABC) in plants are of great importance for growing quality and sufficient crops and sustainable agriculture without sufficient conditions for plant growth. Especially on Arabidopsis, detailed research has been done on ABCs, and it has been discovered that it has a specific mechanism in providing chemodiversity [161]. In the study of Do et al., it has been shown that ABC is necessary for the development and growth of the plant and the interaction of abiotic stress response [162,163]. In the absence of water, ABC transporters take part in closing the stomata and shaping the root so that the water in the soil can be taken up sufficiently. It ensures the preservation of the ionic balance of the cells under salt stress. In addition, it regulates the action of ABA-dependent and independent pathways by triggering TFs (transcription factors) associated with drought stress tolerance and maintains the osmotic balance of the cell. Finally, studies have proven that genes associated with salt stress and drought stress play a role in triggering the expression [164].

XR_0032247259.1 antisense lncRNA biological process is mRNA splicing, via spliceosome. Splicing, which produces multiple transcripts and proteins from the same genes, changes under various environmental conditions that affect plant growth. On splicing, introns included in primary gene transcripts are removed, and exons are ligated to produce competent mRNAs. Cellular component of XR_0032247259.1 is U12-type introns spliceosomal complex. It is from the class of pre-mRNA introns called U12 and contains branching at the specific binding site. The U12 intron type contains about 1% of all intron types [165].

The spliceosome assembly is a highly ordered reaction structure involving ATP molecule hydrolysis and structural rearrangements. Recent studies in plants have found essential roles for the splicing factor in controlling abiotic stress responses. Splicings have modulated stress

responses by targeting the ABA pathway, revealing a new layer in stress tolerance [166]. A Salt stress study on *Arabidopsis* showed that stress changed more than 6000 splicing patterns [167]. In addition, studies have been carried out that more than 1000 genes are associated with splicing in grapes under high temperatures, and RNseq analyses have shown that biotic stress significantly changes splicing in plants [166,168].

XR_182457.4 sense lncRNA is downregulate gene and molecular functions are GTPase activity, and mitotic cell cycle. GTPases play a crucial role in many cell functions and act as timers. GAPs are proteins that accelerate GTPase activity and are vital regulators of G-protein signalling. ROPs, the Rho subfamily whose GTP-binding proteins are encoded in plants, are Rac GTPases. Many recent studies have found that RAC/ROP affects root development, hormone response, response resistance to many biotic stresses, regulating abiotic stress, and opening and closing of stomata [169]. Cao's study on *Arabidopsis* found that *NtRop1* inhibited root growth, increased electrical conductivity, and showed high salt tolerance under high salinity stress [170]. Another significant molecular function of XR_182457.4 gene mitotic cell cycle. The growth and development of plants result from the coordinated expansion of the mitotic cell cycle and the size of the cell. Along with the cell cycle, the endocycle is very important in the development of the plant. It contains most endocycle-linked kinases (CDK) and cyclins (CYC) regulatory proteins in the mitotic cell cycle [171]. A study conducted in sunflowers showed that the decrease in the number of cells in the leaves under drought is dependent on the G1/S transition and that CDKA activity mediates the progression to the S phase. In a similar study, leaf elongation and mitotic cell activity were decreased in mesophyll cells due to decreased CDKA1 activity in wheat seedlings exposed to water deficiency. In the leaves of maize plants exposed to water deficiency, cell division was decreased, and CDKA1 activity was decreased in response to water stress. However, it was observed that the amount of CDKA1 protein did not change; in this case, it was thought that CDKA1 might be responsible for post-translational regulation [172,173]. In summary, signalling molecules regulate the modification of CDKs, causing suppression of their activities. As a result, it stops the cell cycle.

XR_743350.3 antisense lncRNA is upregulate and molecular functions are nucleic acid binding and mRNA binding and nucleic acid binding. The regulation of post-transcriptional RNA metabolism in different plant varieties is provided by RNA-binding proteins (RBPs). Varieties of RBPs include glycine-rich RNA-binding proteins (GRPs). GRPs are thought to

play a role in responding to environmental conditions in plants under stress. It has been investigated under various environmental factors that GRPs are sensitive to stress. In a study conducted under cold stress in rice, GRPs were shown to increase freezing tolerance [174]. The relationship between RBPs and mRNAs may vary according to the stress type of the plant. RBPs provide stabilization of key mRNAs for the continuation of cellular processes under stress, and conversely, RBPs can destabilize unwanted mRNAs [175]. Due to the results of the studies, RBDs are recognized more every day and are used for the development of many agricultural productions, including stress.

XR_002026453.2 sense lncRNA and upregulate molecular gene function is GTPase activity and the same function XR_182457.4 lncRNA. Biological processes are regulation of COPII vesicle coating, endoplasmic reticulum to golgi vesicle-mediated transport, membrane organization and vesicle organization. COPI and COPII coated vesicles are responsible for the transport of proteins. While COPII vesicles are responsible for protein transport from the endoplasmic reticulum (ER) to the Golgi, COPI is responsible for transporting proteins from the Golgi to the ER [176]. Since there is not enough information about the transfer of COPII in plants, Takagi et al. examined the exit of COPII proteins from the ER with microscope images in 2013 [177]. In the study conducted in Arabidopsis, the feature of the COPII mechanism, the presence of multiple COPII was detected with five Sar1, two Sec13, two Sec23, and Sec24 gene duplications. It has been shown that the expression of Sar1a and Sec31a is up-regulated under stress. A 2021 study characterized a distinct population of COPII vesicles in response to abscisic acid in plants. These hormone-induced COPII vesicles are regulated by a plant-specific COPII and carry channels responsible for reducing stress's effects [178].

XR_003244833.1 sense lncRNAs molecular functions are NADP⁺-oxidoreductase activity, alcohol dehydrogenase (NADP⁺) activity, oxidoreductase activity and aldose-6-phosphate reductase (NADPH) activity. NADPH oxidoreductases are ring enzymes involved in electron transfer in the cell membrane. Oxidoreductases carry electrons and mediate a variety of physiological functions. The substrates may be split into two broad categories: NADPH oxidases and NADPH dehydrogenases [179]. Aldose-6-phosphate reductase activities were measured in tomato plants under water deficiency, and A6PR gene expression was examined, and it was observed that increased enzyme activity increased [180].

Results from RNA-Seq data and RT-qPCR analysis are given in figures 4.1 and 4.2. To confirm the RNA-Seq results, three lncRNAs were randomly selected, and RT-qPCR analysis was performed. As expected, XR183413 and XR743190.3 lncRNAs were downregulated, while XR003244833.1 was upregulated. Results from RT-qPCR analysis confirmed the reliability of RNA-seq analysis.

In summary, in this study, which was conducted on tomatoes for the drought problem that has been increasing in recent years, chlorophyll amount, soil moisture, root and shoot length, water content, soluble protein content and lipid peroxidation analyses were performed. According to the experimental results, there was no big decrease in water content in either the Falcon or SC2121 varieties. This might be caused by the drought stress that applied for 1 week was not sufficient for Falcon and SC2121 cultivars. Protein content and root length show that Falcon is stressed and responding. SC2121 appears to be shutting down and maybe waiting for conditions to improve i.e. waiting for water. Since the membrane structure of the plant deteriorate under drought stress, the amount of MDA increases, but according to our result, the amount of MDA decreases. This situation may indicate that sufficient drought application was not applied to the extent that the membrane would be damaged. According to the NGS analysis result, the lncRNAs obtained show drought-related and stress-related expression. All of the lncRNAs that show differential expression are found to both Falcon and SC2121 variants.

5. CONCLUSION

The study performed chlorophyll content, root and shoot length, water content, protein content, and lipid peroxidation analyses of two different tomato cultivars under drought stress. According to the results obtained, it was seen that the responses of plants under drought stress were the similar to others in the literature. While chlorophyll amount decreased in SC2121 cultivar, chlorophyll amount increased towards the end of the seventh day in Falcon species and Falcon is responding well to stress. The root length results, while the root length of Falcon species increases in drought conditions, the root length of SC2121 decreases. A similar change is seen in the lipid peroxidation analysis. The amount of MDA decreased slightly in the Falcon cultivar, while the amount of MDA was significantly reduced in the SC2121 cultivar. Considering these results, Falcon cultivar can be drought resistant, but when the water content and protein content results are considered, it is seen that the results are opposite. Undoubtedly, comprehensive studies must evaluate tomato varieties' physiological and biochemical properties.

According to the next generation sequencing analysis, 269 differentially expressed lncRNAs were identified. While 124 of these lncRNAs were up-regulated, 145 were down-regulated. Among the lncRNAs showing differential expression, the 12 with the greatest degree of differential expression were selected and their molecular functions and biological processes were investigated. It was determined that the selected lncRNAs were directly related to drought stress. This is the first report of lncRNA research on Falcon and SC2121 tomato cultivars under drought. This study's findings may be precursor since they light up the mechanisms regulating gene expression and the amounts of lncRNA expression in various tomato cultivars. The functions, expression patterns, and localizations of lncRNAs for the cultivation of drought-resistant vegetables in the future are only beginning to be understood. Consequently, this study may bring beneficial to understand the evolutionary relationships of lncRNAs in the future.

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